

## PATENT COOPERATION TREATY

PCT

## NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

United States Patent and Trademark  
Office  
(Box PCT)  
Crystal Plaza 2  
Washington, DC 20231  
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year)

14 October 1996 (14.10.96)

International application No.

PCT/AU96/00094

Applicant's or agent's file reference

PN1457 EJH/EK

International filing date (day/month/year)

22 February 1996 (22.02.96)

Priority date (day/month/year)

02 March 1995 (02.03.95)

Applicant

HAYWARD, Nicholas, Kim et al

1. The designated Office is hereby notified of its election made:



in the demand filed with the International Preliminary Examining Authority on:

30 September 1996 (30.09.96)



in a notice effecting later election filed with the International Bureau on:

2. The election



was



was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO  
34, chemin des Colombettes  
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

R. Raissi

Telephone No.: (41-22) 730.91.11

## PCT

## INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference PN 1457 EJH/EK	<b>FOR FURTHER ACTION</b> see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. <b>PCT/AU 96/00094</b>	International filing date ( <i>day/month/year</i> ) 22 February 1996	(Earliest) Priority Date ( <i>day/month/year</i> ) 02 March 1995
Applicant (1) <b>AMRAD OPERATIONS PTY LTD</b> (2) <b>HAYWARD, Nicholas Kim and WEBER, Gunther</b>		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of **4** sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. ☐ **Certain claims were found unsearchable** (See Box I)
2. ☐ **Unity of invention is lacking** (See Box II)
3. ☒ The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
  - ☒ filed with the international application
  - ☐ furnished by the applicant separately from the international application,
    - ☐ but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed
  - ☐ transcribed by this Authority
4. With regard to the **title**,
  - ☒ the text is approved as submitted by the applicant.
  - ☐ the text has been established by this Authority to read as follows:
5. With regard to the **abstract**,
  - ☒ the text is approved as submitted by the applicant
  - ☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.
6. The figure of the **drawings** to be published with the abstract is:
 

Figure No.

  - ☐ as suggested by the applicant.
  - ☐ because the applicant failed to suggest a figure
  - ☐ because this figure better characterises the invention
  - ☒ None of the figures

**A. CLASSIFICATION OF SUBJECT MATTER**Int Cl<sup>6</sup>: C12N 15/12; C07K 14/475; A61K 037/02

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

WPAT AND CHEM ABS

SEE DETAILS IN ELECTRONIC DATABASE BOX BELOW

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched  
USPM, JAPIO

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

DERWENT WPAT, USPM, JAPIO DATABASES; KEYWORDS: MVRF OR HVRF OR SOM 1: OR SOM X: OR [VASOACTIVE ( ) PERMEABILITY ( ) FACTOR#] OR VEGF: OR VEGF OR VRF: OR VRF OR [GROWTH ( ) FACTOR (5N) (VASCULAR OR ENDOTHEL:)]  
CHEMICAL ABSTRACTS DATABASE; KEYWORDS: AS ABOVE**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	AU 60798/90 (CALIFORNIA BIOTECHNOLOGY INC) published 21 February 1991	1-41
X	AU 56574/90 (GENENTECH, INC.) published 15 November 1990	1-41
P,X	AU 73941/94 (HUMAN GENOME SCIENCES, INC.) published 14 September 1995	1-41



Further documents are listed in the continuation of Box C



See patent family annex

* Special categories of cited documents:		
"A"	document defining the general state of the art which is not considered to be of particular relevance	"I" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E"	earlier document but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"I."	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P"	document published prior to the international filing date but later than the priority date claimed	

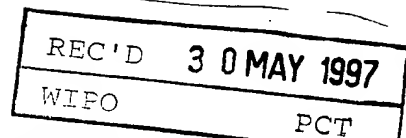
Date of the actual completion of the international search 03 June 1996	Date of mailing of the international search report 5 <sup>TH</sup> JUNE 1996.
Name and mailing address of the ISA/AU AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No.: (06) 285 3929	Authorized officer  <b>ARATI SARDANA</b> Telephone No.: (06) 283 2627

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5073492 (Chung-Ho Chen and Sumi C. Chen) published 17 December 1991	34
X	Biochemical and Biophysical Research Communications (1992), Vol 183, No. 3, pg 1167-1174 (Weindel K. et al.) "AIDS-ASSOCIATED KAPOSI'S SARCOMA CELLS IN CULTURE EXPRESS VASCULAR ENDOTHELIAL GROWTH FACTOR". See whole Article.	1-41
X	Biochemical and Biophysical Research Communications (1989), Vol 165, No. 3 pg 1198-1206 (Edmund Tischer et al.) "VASCULAR ENDOTHELIAL GROWTH FACTOR : A NEW MEMBER OF THE PLATELET-DERIVED GROWTH FACTOR GENE FAMILY". See whole Article.	1-41
X	Journal of Virology (1994), Vol 68, No. 1 pg 84-92 (David J. Lyttle et al.) "HOMOLOGS OF VASCULAR ENDOTHELIAL GROWTH FACTOR ARE ENCODED BY THE POX VIRUS ORF VIRUS". See whole Article.	1-41
X	Methods in Enzymology (1991), vol 198, pg 391-405 (Ferrara Napoliana et al.) "PURIFICATION AND CLONING OF VASCULAR ENDOTHELIAL GROWTH FACTOR SECRETED BY PITUITARY FOLLICULOCELLULAR CELLS". See whole Article	1-41
X	The Journal of Biological Chemistry (1991), vol 266, No. 18 pg 11947-11954 (Edmund Tischer et al.) "THE HUMAN GENE FOR VASCULAR ENDOTHELIAL GROWTH FACTOR". See whole Article	1-41
P,X	Biochemical and Biophysical Research Communications (1996), Vol 220, No. 1 pg 147-52 (Lagercrantz J et al) "EXPRESSION OF THE VEGF-RELATED FACTOR GENE IN PRE- AND POSTNATAL MOUSE". See whole Article	33
P,X	Biochimica et Biophysica Acta (1995), Vol. 1260 No. 2 pg 235-9 (Sharma Hari S et al.) "NUCLEOTIDE SEQUENCE AND EXPRESSION OF THE PORCINE VASCULAR ENDOTHELIAL GROWTH FACTOR". See whole Article.	1-41
X	DEVELOPMENT (1992), Vol 114, pg 521-532 (Breier G et al.) "EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR DURING EMBRYONIC ANGIOGENESIS AND ENDOTHELIAL CELL DIFFERENTIATION". See whole Article	1-41
X	Molecular Endocrinology (1991), Vol 5 No. 12, pg 1806-1814 (Houck Keith A et al.) "THE VASCULAR ENDOTHELIAL GROWTH FACTOR FAMILY: IDENTIFICATION OF A FOURTH MOLECULAR SPECIES AND CHARACTERIZATION OF ALTERNATIVE SPLICING OF RNA". See whole Article	1-41



This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report				Patent Family Member			
AU	56574/90	US IL WO	5332671 94128 9013649	CA JP	2054699 4505705	EP NZ	471754 233451
AU	60798/90	US JP	5194596 5501350	CA WO	2063810 9102058	EP US	484401 5219739
AU	73941/94	WO	9524473				
US	5073492	NONE					



Applicant's or agent's file reference 1807660/EJH/EK	<b>FOR FURTHER ACTION</b>	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International application No. <b>PCT/AU 96/00094</b>	International filing date 22 February 1996	Priority Date 02 March 1995
International Patent Classification (IPC) or national classification and IPC  <b>Int. Cl.<sup>6</sup> C12N 15/12; C07K 14/475; A61K 037/02</b>		
Applicant (1) <b>AMRAD OPERATIONS PTY LTD</b> (2) <b>HAYWARD, Nicholas Kim and WEBER, Gunther</b>		

1.	This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
2.	This REPORT consists of a total of <b>8</b> sheets, including this cover sheet.  <input type="checkbox"/> This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).  These annexes consist of a total of      sheet(s).
3.	This report contains indications relating to the following items:  I <input checked="" type="checkbox"/> Basis of the report II <input type="checkbox"/> Priority III <input type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability IV <input checked="" type="checkbox"/> Lack of unity of invention V <input checked="" type="checkbox"/> Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement VI <input type="checkbox"/> Certain documents cited VII <input checked="" type="checkbox"/> Certain defects in the international application VIII <input checked="" type="checkbox"/> Certain observations on the international application

Date of submission of the demand 30 September 1996	Date of completion of the report 20 May 1997
Name and mailing address of the IPEA/AU AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No. (06) 285 3929	Authorized Officer <i>Arati Sardana</i> <b>ARATI SARDANA</b> Telephone No. (06) 283 2627

**I. Basis of the report**

1. This report has been drawn on the basis of *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.)*:

☒ the international application as originally filed.

☐ the description,        pages , as originally filed,  
                                 pages , filed with the demand,  
                                 pages , filed with the letter of ,  
                                 pages , filed with the letter of .

☐ the claims,        Nos. , as originally filed,  
                                 Nos. , as amended under Article 19,  
                                 Nos. , filed with the demand,  
                                 Nos. , filed with the letter of ,  
                                 Nos. , filed with the letter of .

☐ the drawings,        sheets/fig , as originally filed,  
                                 sheets/fig , filed with the demand,  
                                 sheets/fig , filed with the letter of ,  
                                 sheets/fig , filed with the letter of .

2. The amendments have resulted in the cancellation of:

☐ the description,        pages

☐ the claims,        Nos.

☐ the drawings,        sheets/fig

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

4. Additional observations, if necessary:

**IV. Lack of unity of invention**

1. In response to the invitation to restrict or pay additional fees the applicant has:

- ☐ restricted the claims.
- ☐ paid additional fees.
- ☐ paid additional fees under protest.
- ☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

- ☒ complied with.
- ☐ not complied with for the following reasons:

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

- ☒ all parts.
- ☐ the parts relating to claims Nos.

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement****1. Statement**

Novelty (N)	Claims	NONE	YES
	Claims	1-41	NO
Inventive step (IS)	Claims	NONE	YES
	Claims	1-41	NO
Industrial applicability (IA)	Claims	1-14	YES
	Claims	NONE	NO

**2. Citations and explanations****Document 1**

AU 60798/90 (CALIFORNIA BIOTECHNOLOGY INC) published 21 February 1991

Figure 6 in document 1 discloses isolated cDNA sequences encoding bVEGF<sub>120</sub> and bVEGF<sub>164</sub> both these sequences show at least 15% similarity and 5% dissimilarity to the sequence set forth in sequence ID#2.

**Document 2**

AU 56574/90 (GENENTECH, INC) published 15 November 1990

Figures 2 and 12 disclose amino acid sequences having at least 15% similarity and 5% dissimilarity to the sequence set forth in sequence ID#2.

**Document 3**

Biochemical and Biophysical Research Communication (1989), Volume 165, No. 3 page 1198-1206 (Edmund Tischer et al.) "VASCULAR ENDOTHELIAL GROWTH FACTOR: A NEW MEMBER OF THE PLATELET-DERIVED GROWTH FACTOR GENE FAMILY". See whole Article.

Sequence of figure 3 in document 3 has at least 15% similarity and 5% dissimilarity to the sequence set forth in sequence ID#2.

**Document 4**

Journal of Virology (1994), Volume 68, No. 1 page 84-92 (David J Lyttle et al.) "HOMOLOGS OF VASCULAR ENDOTHELIAL GROWTH FACTOR ARE ENCODED BY THE POX VIRUS ORF VIRUS". See whole Article.

Figure 5 of document 4 discloses Orf virus NZ7 and NZ2 genes encoding polypeptides which are at least 15% similar and 5% dissimilar to the sequence set forth in sequence ID#2.

continued

**Supplemental Box**

(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of : BOX V.2

**Document 5**

Methods in Enzymology (1991), Volume 198, page 391-405 (Ferrara Napoliana et al.) "PURIFICATION AND CLONING OF VASCULAR ENDOTHELIAL GROWTH FACTOR SECRETED BY PITUITARY FOLLICULOCELLULAR CELLS". See whole Article.

Figure 1 in document 5 discloses bovine VEGF cDNA clone encoding amino acid sequence that is at least 15% similar and 5% dissimilar to the sequence set forth in sequence ID#2.

**Document 6**

The Journal of Biological Chemistry (1991), Volume 266, No. 18 page 11947-11954 (Edmund Tischer et al.) "THE HUMAN GENE FOR VASCULAR ENDOTHELIAL GROWTH FACTOR". See whole Article.

Figure 2 of document 6 discloses PCR products PCR-1 and PCR-3 having sequences that are at least 15% similar and 5% dissimilar to the sequence set forth in sequence ID#2.

**Document 7**

DEVELOPMENT (1992), Volume 114, page 521-532 (Breier G et al.) "EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR DURING EMBRYONIC ANGIOGENESIS AND ENDOTHELIAL CELL DIFFERENTIATION". See whole Article.

Figure 3 of document 7 discloses mouse, bovine and rat VEGF protein sequences that are at least 15% similar and 5% dissimilar to the sequence set forth in sequence ID#2.

**Document 8**

Molecular Endocrinology (1991), Volume 5 No. 12, page 1806-1814 (Houck Keith A et al.) "THE VASCULAR ENDOTHELIAL GROWTH FACTOR FAMILY: IDENTIFICATION OF A FOURTH MOLECULAR SPECIES AND CHARACTERISATION OF ALTERNATIVE SPLICING OF RNA". See whole Article.

Figure 2 in document 8 discloses comparison between multiple VEGF molecular species VEGF 121, VEGF 189 and VEGF 206 resulting from alternative splicing of VEGF RNA. The amino acid sequences of these alternative species are 15% similar and 5% dissimilar to the sequence set forth in sequence ID#2.

**Documents 9 and 10**

US Patent Numbers: 5219739 and 5194596 disclose amino acid sequences of human and bovine VEGF<sub>121</sub> and VEGF<sub>120</sub> species. These sequences are at least 15% similar and 5% dissimilar to the sequence set forth in sequence ID#2.

**Document 11**

Science, Volume 246, 8 December 1989 page 1309-12.

continued

**Supplemental Box**

(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: V.2

Document 11 in figure 1 discloses a VEGF species that is 15% similar and 5% dissimilar to the sequence set forth in sequence ID#2.

Documents 12 and 13

Science Volume 264, 8 December 1989 page 1306-1309 and US Patent number 5332671.

Both these documents disclose bovine VEGF<sub>164</sub> amino acid species that is at least 15% similar and 5% dissimilar to the sequence ID#2.

Document 14

Biochimica et Biophysica Acta 1260 (1995) 235-238.

Document 14 discloses Porcine vascular endothelial growth factor that has an amino acid sequence that is 15% similar and 5% dissimilar to the sequence set forth in sequence ID#2.

Claims 1-41 as drafted include within their scope documents disclosing proteinaceous molecules or recombinant molecules comprising amino acid sequence having at least 15% similarity and 5% dissimilarity to the sequence set forth in sequence ID No. 2 and exhibit at least one property in common with VEGF.

All the above documents numbered 1-14 disclose proteins having at least 15% amino acid sequence similarity and 5% dissimilarity to the sequence set forth in sequence ID No. 2 and exhibit at least one property in common with VEGF. Therefore all these documents fall within the scope of claims 1-41 rendering them not novel and not inventive.

Claim 33 defining a nucleic acid molecule encoding murine homologue of human VEGF comprising nucleotide sequence of Figure 9 lacks an inventive step in the light of Document 7 disclosing mouse and rat VEGF protein sequences.

The citation discloses mouse and rat VEGF genes and similarity to the sequence of Figure 9. Common General Knowledge of Skilled addressee would use probes based on known sequence to get murine homologue of human VEGF. Therefore claim 33 lacks an inventive step.

**VII. Certain defects in the international application**

The following defects in the form or contents of the international application have been noted:

Last two claims have been numbered 41.



**VIII. Certain observations on the international application**

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Claims defining portions, parts, fragments and derivatives of the present sequences are indeterminate in scope.



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>C12N 15/12, C07K 14/475, A61K 37/02</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 96/27007</b> <b>(43) International Publication Date:</b> 6 September 1996 (06.09.96)
<b>(21) International Application Number:</b> PCT/AU96/00094 <b>(22) International Filing Date:</b> 22 February 1996 (22.02.96) <b>(30) Priority Data:</b> PN 1457 ✓ 2 March 1995 (02.03.95) AU PN 6647 ✓ 20 November 1995 (20.11.95) AU PN 7274 ✓ 22 December 1995 (22.12.95) AU <b>(71) Applicant (for all designated States except US):</b> AMRAD OPERATIONS PTY. LTD. [AU/AU]; 17-27 Cotham Road, Kew, VIC 3101 (AU). <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> HAYWARD, Nicholas, Kim [AU/AU]; 13 Prince Street, Paddington, QLD 4064 (AU). WEBER, Gunther [SE/AU]; 17-27 Cotham Road, Kew, VIC 3101 (AU). GRIMMOND, Sean [AU/AU]; 210 Swann Road, Taringa, QLD 4068 (AU). NORDEN-SKJOLD, Magnus [SE/SE]; Koltrastvagen 19, S-161 37 Stockholm (SE). LARSSON, Catharina [SE/SE]; Dannemoragatan 10m 3tr, S-113 44 Stockholm (SE). <b>(74) Agents:</b> HUGHES, E., John, L. et al.; Davies Collison Cave, 1 Little Collins Street, Melbourne, VIC 3000 (AU).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> A NOVEL GROWTH FACTOR AND A GENETIC SEQUENCE ENCODING SAME		
<b>(57) Abstract</b> <p>The present invention relates generally to an isolated molecule having vascular endothelial growth factor-like properties and to a genetic sequence encoding same. The molecule will be useful in the development of a range of therapeutics and diagnostics useful in the treatment, prophylaxis and/or diagnosis of conditions requiring enhanced or diminished vasculature and/or vascular permeability. The molecule of the present invention is also a useful effector of primary and central neurons and is capable of inducing astroglial proliferation.</p>		

A NOVEL GROWTH FACTOR AND A GENETIC SEQUENCE  
ENCODING SAME

5 The present invention relates generally to an isolated molecule having vascular endothelial growth factor-like properties and to a genetic sequence encoding same. The molecule will be useful in the development of a range of therapeutics and diagnostics useful in the treatment, prophylaxis and/or diagnosis of conditions requiring enhanced or diminished vasculature and/or vascular permeability. The molecule of the present  
10 invention is also a useful effector of primary and central neurons and is capable of inducing astroglial proliferation.

Bibliographic details of the publications referred to by author in this specification are collected at the end of the description. Sequence Identity Numbers (SEQ ID NOs.) for  
15 the nucleotide and amino acid sequences referred to in the specification are defined following the bibliography.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to  
20 imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

Vascular endothelial growth factor (hereinafter referred to as "VEGF"), also known as vasoactive permeability factor, is a secreted, covalently linked homodimeric glycoprotein  
25 that specifically activates endothelial tissues (Senger *et al.*, 1993). A range of functions have been attributed to VEGF such as its involvement in normal angiogenesis including formation of the corpus luteum (Yan *et al.*, 1993) and placental development (Sharkey *et al.*, 1993), regulation of vascular permeability (Senger *et al.*, 1993), inflammatory angiogenesis (Sunderkotter *et al.*, 1994) and autotransplantation (Dissen *et al.*, 1994) and  
30 human diseases such as tumour promoting angiogenesis (Folkman & Shing, 1992), rheumatoid arthritis (Koch *et al.*, 1994) and diabetes related retinopathy (Folkman & Shing, 1992).

- 2 -

VEGF is, therefore, an important molecule making it a potentially valuable target for research into therapeutics, prophylactics and diagnostic agents based on VEGF or its activities. There is also a need to identify homologues or otherwise related molecules for use as an alternative to VEGF or in conjunction with VEGF.

5

In work leading up to the present invention, the inventors sought the multiple endocrine neoplasia type I susceptibility gene (MEN1). Surprisingly, the inventors discovered that a genetic sequence excluded as a candidate for the MEN1 gene was nevertheless a new growth factor having some similarity to VEGF. Furthermore, the growth factor of the present invention is an effector molecule for primary and central neurons.

10

Accordingly, one aspect of the present invention comprises a biologically isolated proteinaceous molecule comprising a sequence of amino acids which:

15

- (i) is at least about 15% similar to the amino acid sequence set forth in SEQ ID NO:2; and
- (ii) is at least 5% dissimilar to the amino acid sequence set forth in SEQ ID NO:2.

Another aspect of the present invention provides a biologically isolated proteinaceous molecule having the following characteristics:

- 20 (i) comprises an amino acid sequence having at least about 15% similarity but at least about 5% dissimilarity to all or part of the amino acid sequence set forth in SEQ ID NO:2;
- (ii) exhibits at least one property in common with VEGF.

25 A related aspect of the present invention contemplates a biologically isolated proteinaceous molecule having the following characteristics:

- (i) comprises an amino acid sequence having at least about 15% similarity but at least about 5% dissimilarity to the amino acid sequence set forth in SEQ ID NO:2;
- 30 (ii) exhibits at least one of the following properties:
  - (a) ability to induce proliferation of vascular endothelial cells;
  - (b) ability to interact with *flt-1/flk-1* family of receptors;

- 3 -

- (c) ability to induce cell migration, cell survival and/or an increase in intracellular levels of alkaline phosphatase.

By "biologically isolated" is meant that the molecule has undergone at least one step of purification from a biological source. Preferably, the molecule is also biologically pure meaning that a composition comprises at least about 20%, more preferably at least about 40%, still more preferably at least about 65%, even still more preferably at least about 80-90% or greater of the molecule as determined by weight, activity or other convenient means, relative to other compounds in the composition. Most preferably, the molecule is sequencably pure.

Another preferred aspect of the present invention provides the molecule in recombinant form.

According to this aspect of the present invention, there is provided a recombinant molecule comprising a sequence of amino acids which:

- (i) is at least about 15% similar to the amino acid sequence set forth in SEQ ID NO:2; and
- (ii) is at least 5% dissimilar to the amino acid sequence set forth in SEQ ID NO:2.

A related aspect of the present invention is directed to a recombinant molecule having the following characteristics:

- (i) comprises an amino acid sequence having at least about 15% similarity but at least about 5% dissimilarity to all or part of the amino acid sequence set forth in SEQ ID NO:2;
- (ii) exhibits at least one property in common with VEGF.

A further related aspect of the present invention contemplates a recombinant molecule having the following characteristics:

- (i) comprises an amino acid sequence having at least about 15% similarity but at least about 5% dissimilarity to the amino acid sequence set forth in SEQ ID NO:2;

- (ii) exhibits at least one of the following properties:
- (a) ability to induce proliferation of vascular endothelial cells;
  - (b) ability to interact with *flt-1/flk-1* family of receptors;
  - (c) ability to induce cell migration, cell survival and/or an increase in intracellular levels of alkaline phosphatase.

The present invention also contemplates genomic or partial genome clones encoding a proteinaceous molecule having at least about 15% amino acid similarity but at least about 5% dissimilarity to SEQ ID NO:1.

10

The amino acid sequence set forth in SEQ ID NO:2 corresponds to human VEGF (referred to herein as "VEGF<sub>165</sub>"). Accordingly, the molecule of the present invention is VEGF-like or is a homologue of VEGF but comprises an amino acid sequence which is similar but non-identical to the amino sequence of VEGF. Although the present invention is exemplified using a human VEGF-like molecule, this is done with the understanding that the instant invention contemplates the homologous molecule and encoding sequence from other mammals such as livestock animals (e.g. sheep, pigs, horses and cows), companion animals (e.g. dogs and cats) and laboratory test animals (e.g. mice, rats, rabbits and guinea pigs) as well as non-mammals such as birds (e.g. poultry birds), fish and reptiles. In a most preferred embodiment, the VEGF-like molecule is of human origin and encoded by a gene located at chromosome 11q13. The present invention extends, therefore, to the genomic sequence or part thereof encoding the subject VEGF-like molecule.

Preferably, the percentage similarity is at least about 30%, more preferably at least about 40%, still more preferably at least about 50%, still even more preferably at least about 60-70%, yet even more preferably at least about 80-95% to all or part of the amino acid sequence set forth in SEQ ID NO:2.

In a particularly preferred embodiment, the VEGF-like molecule of the present invention comprises a sequence of amino acids as set forth in SEQ ID NO:4 or is a part, fragment, derivative or analogue thereof. Particularly preferred similarities include about 19-20%,

- 5 -

and 29-30%. Reference herein to derivatives also includes splice variants. Accordingly, the present invention extends to splice variants of SOM175. Examples of splice variants contemplated by the present invention include but are not limited to variants with an amino acid sequence substantially as set forth in at least one of SEQ ID NO:6, SEQ ID  
5 NO:8 and/or SEQ ID NO:10 or mutants or derivatives or further splice variants thereof.

Another embodiment provides a recombinant molecule having the following characteristics:

- 10 (i) an amino acid sequence substantially as set forth in SEQ ID NO:4 or having at least about 15% similarity to all or part thereof provided that said amino acid sequence is at least about 5% dissimilar to all or part of the amino acid sequence set forth in SEQ ID NO:2;
- (ii) exhibits at least one biological property in common with VEGF.

15 Another embodiment provides a recombinant molecule having the following characteristics:

- 20 (i) an amino acid sequence substantially as set forth in SEQ ID NO:6 or having at least about 15% similarity to all or part thereof provided that said amino acid sequence is at least about 5% dissimilar to all or part of the amino acid sequence set forth in SEQ ID NO:2;
- (ii) exhibits at least one biological property in common with VEGF.

Another embodiment provides a recombinant molecule having the following characteristics:

- 25 (i) an amino acid sequence substantially as set forth in SEQ ID NO:8 or having at least about 15% similarity to all or part thereof provided that said amino acid sequence is at least about 5% dissimilar to all or part of the amino acid sequence set forth in SEQ ID NO:2;
- (ii) exhibits at least one biological property in common with VEGF.

30

Another embodiment provides a recombinant molecule having the following characteristics:

- 6 -

- (i) an amino acid sequence substantially as set forth in SEQ ID NO:10 or having at least about 15% similarity to all or part thereof provided that said amino acid sequence is at least about 5% dissimilar to all or part of the amino acid sequence set forth in SEQ ID NO:2;
- 5 (ii) exhibits at least one biological property in common with VEGF.

Such properties of VEGF include at least one of:

- (a) ability to induce proliferation of vascular endothelial cells;
- (b) an ability to interact with *flt-1/flk-1* family of receptors;
- 10 (c) an ability to induce cell migration, cell survival and/or an increase in intracellular levels of alkaline phosphatase.

In accordance with the present invention, a preferred similarity is at least about 40%, more preferably at least about 50% and even more preferably at least about 65% similarity.

15

Still a further aspect of the present invention contemplates a peptide fragment corresponding to a portion of the amino acid sequence set forth in SEQ ID NO:4 or a splice variant thereof such as set forth in SEQ ID NO:6, SEQ ID NO:8 or SEQ ID NO:10 or a chemical equivalent thereof. The biologically isolated or recombinant molecule of the present invention may be naturally glycosylated or may comprise an altered glycosylation pattern depending on the cells from which it is isolated or synthesised. For example, if produced by recombinant means in prokaryotic organisms, the molecule would be non-glycosylated. The molecule may be a full length, naturally occurring form or may be a truncated or otherwise derivatised form.

20

25

Yet another aspect of the present invention is directed to a nucleic acid molecule encoding the VEGF-like molecule herein described. More particularly, the present invention provides a nucleic acid molecule comprising a sequence of nucleotides substantially as set forth in SEQ ID NO:3 or having at least 15% similarity to all or part thereof or being capable of hybridising under low stringency conditions to a reverse complement of the nucleotide sequence as set forth in SEQ ID NO:3 provided that the

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- 7 -

nucleic acid sequence having at least 15% similarity but at least 30% dissimilarity to the nucleotide sequence as set forth in SEQ ID NO:3. The nucleotide sequence set forth in SEQ ID NO:3 is also referred to herein as "SOM175". Preferably, the percentage dissimilarity is about 35%, more preferably about 39% and even more preferably about 40-50% or greater.

For the purposes of defining the level of stringency, reference can conveniently be made to Sambrook *et al* (1989) at pages 9.47-9.51 which is herein incorporated by reference where the washing steps disclosed are considered high stringency. A low stringency is defined herein as being in 4-6X SSC/0.1-0.5% w/v SDS at 37-45°C for 2-3 hours. Depending on the source and concentration of nucleic acid involved in the hybridisation, alternative conditions of stringency may be employed such as medium stringent conditions which are considered herein to be 1-4X SSC/0.25-0.5% w/v SDS at  $\geq 45^{\circ}\text{C}$  for 2-3 hours or high stringent conditions considered herein to be 0.1-1X SSC/0.1% w/v SDS at 60°C for 1-3 hours.

The present invention further contemplates a nucleic acid molecule which encodes a VEGF-like molecule as hereinbefore described having at least 15% nucleotide sequence homology to SEQ ID NO:3. Preferred levels of homology include at least about 40%, more preferably around 60-70%.

The present invention is further directed to the murine homologue of human VEGF (referred to herein as "mVRF"). The mVRF has approximately 85% identity and 92% conservation of amino acid residues over the entire coding region compared to human VEGF. The mVRF is encoded by a nucleic acid molecule comprising a nucleotide sequence substantially as set forth in Figure 9.

The VEGF-like molecule of the present invention will be useful in the development of a range of therapeutic and/or diagnostic applications alone or in combination with other molecules such as VEGF. The present invention extends, therefore, to pharmaceutical compositions comprising the VEGF-like molecule or parts, fragments, derivatives, homologues or analogues thereof together with one or more pharmaceutically acceptable

carriers and/or diluents. Furthermore, the present invention extends to vectors comprising the nucleic acid sequence set forth in SEQ ID NO:3 or having at least about 15%, more preferably about 40% and even more preferably around 60-70% similarity thereto but at least 30% and more preferably around 39% dissimilarity thereto and host cells comprising same. In addition, the present invention extends to ribozymes and antisense molecules based on SEQ ID NO:3 as well as neutralizing antibodies to the VEGF-like molecule. Such molecules may be useful in ameliorating the effects of, for example, over expression of VEGF-like genes leading to angiogenesis or vascularization of tumours.

10

Another aspect of the present invention contemplates a method of inducing astroglial proliferation in a mammal, said method comprising administering to said mammal an effective amount of a recombinant proteinaceous molecule having the characteristics:

- (i) comprises an amino acid sequence having at least about 15% similarity but at least about 5% dissimilarity to the sequence set forth in SEQ ID NO:2;
- (ii) exhibits at least one property in common with vascular endothelial growth factor (VEGF),

said administration being for a time and under conditions sufficient to induce astroglial proliferation.

20

Preferably, the recombinant proteinaceous molecule comprises the amino acid sequence set forth in SEQ ID NO:3 or SEQ ID NO:6.

A further aspect of the present invention provides a method of promoting neural survival and/or proliferation in a mammal, said method comprising administering to said mammal an effective amount of a recombinant proteinaceous molecule having the characteristics:

- (i) comprises an amino acid sequence having at least about 15% similarity but at least about 5% dissimilarity to the sequence set forth in SEQ ID NO:2;
- (ii) exhibits at least one property in common with vascular endothelial growth factor (VEGF),

30

said administration being for a time and under conditions sufficient to induce astroglial proliferation.

Preferably, the recombinant proteinaceous molecule comprises the amino acid sequence  
5 set forth in SEQ ID NO:3 or SEQ ID NO:6.

The present invention also contemplates antibodies to the VEGF-like molecule or nucleic acid probes to a gene encoding the VEGF-like molecule which are useful as diagnostic agents.

10

The present invention is further described by reference to the following non-limiting Figures and/or Examples.

In the Figures:

15

**Figure 1** Nucleotide sequence [SEQ ID NO:1] and corresponding amino acid sequence [SEQ ID NO:2] of VEGF<sub>165</sub>.

**Figure 2** Nucleotide sequence [SEQ ID NO:3] and corresponding amino acid  
20 sequence [SEQ ID NO:4] of SOM175.

**Figure 3** Results of BLAST search with SOM175 protein sequence.

**Figure 4** BESTFIT alignment of VEGF cDNA and SOM175 cDNA.

25

**Figure 5** Multiple alignment of VEGF<sub>165</sub> with SOM175 and its splice variants at the nucleotide level.

**Figure 6** Multiple alignment of VEGF<sub>165</sub> with SOM175 and its splice variants at  
30 the amino acid level.

**Figure 7** Diagrammatic representation of SOM175 and its splice variants.

**Figure 8(a)** Diagrammatic representation of genomic structure of human SOM175 genomic showing exon/intron map.

**Figure 8(b)** Diagrammatic representation of genomic structure of human SOM175 showing exon/intron boundaries.

**Figure 9** Nucleotide and predicted peptide sequences derived from mVRF cDNA clones. Numbering of nucleotides are given on the left, starting from the A of the initiation codon. Amino acids are numbered on the right, starting from the first residue of the predicted mature protein after the putative signal peptide has been removed. The alternately spliced region is double underlined and the resulting peptide sequence from each mRNA is included. A potential polyadenylation signal is indicated in boldface. Start and stop codons of mVRF<sub>167</sub> and mVRF<sub>186</sub> are underlined and a polymorphic AC repeat in the 3' UTR is indicated by a stippled box. The positions of intron/exons boundaries are indicated by arrowheads.

**Figure 10** BESTFIT alignments of human and murine VRF protein isoforms. A: mVRF<sub>167</sub> and hVRF<sub>167</sub>. B: mVRF<sub>186</sub> and hVRF<sub>186</sub> from the point where the sequences diverge from the respective 167 amino acid isoforms. Amino acid identities are marked with vertical bars and conserved amino acids with colons. An arrow marks the predicted signal peptide cleavage site of human and mouse VRF.

**Figure 11** BESTFIT alignment of mVRF<sub>167</sub> and mVEGF<sub>188</sub> (Breier *et al*, 1992) peptide sequences. An arrow marks the signal peptide cleavage site of mVEGF. Identical amino acids are indicated by vertical bars and conservative substitutions by colons. Numbering of amino acids is as described in the legend to Figure 9.

**Figure 12** Comparison of gene structure between VRF (a generic VRF gene is shown since the intron/exon organisation of the mouse and human homologues is almost identical) and other members of the human VEGF/PIGF/PDGF gene family. Exons are represented by boxes. Protein coding regions and untranslated regions are shown by filled and open sections respectively. The hatched region in VRF indicates the

additional 3' UTR sequence formed by alternate splicing of the VRF<sub>186</sub> isoform. Potential alternate splice products of each gene are shown.

**Figure 13** Autoradiogram of a Northern blot of total RNA from various adult mouse tissues (as indicated) hybridised with an mVRF cDNA clone. A major transcript of 1.3 kb was detected in all samples.

**Figure 14** Film autoradiographs (A-C) and dark-field micrographs (D-E) illustrating the expression pattern of mVRF and mRNA in the mouse. In the E14 mouse embryo (A) positive signals are present over the developing heart (Ha) and cerebral cortex (Cx). A low background signal is also present over other tissues in the section. In the E17 embryo (B) and the heart (Ha) is clearly visible due to a strong hybridisation signal. An equally strong signal is present over brown adipose tissue (Fa) in the back and around the thoracic cage. A moderate hybridisation signal is present over the spinal cord (SC) and the tongue (T). The background signal is reduced compared with the E14 embryo. In the young adult mouse (C-D), positive signals are present over the heart (Ha) and adipose tissue (Fa) around the thoracic cage, while, for example, the lungs (Lu) are unlabeled). The hybridisation signal over the heart is evenly distributed over the entire left ventricle, including papillary muscles (D). In the E17 heart hybridised with an excess of cold probe, no positive signal is present (E). Scale bars = 0.5 mm (A), 1.2 mm (B), 1 mm (C), 0.3 mm (D), 0.1 mm (E).

**Figure 15** Dark - (A and C) and bright-field (B and D) micrographs showing mVRF mRNA expression in mouse adipose tissue (A-B) and spinal cord (C-D). A strong hybridisation signal is present over fat (A), as shown by the strong labeling in Sudan black stained sections (B). A weak signal is present also in skeletal muscle (M in A-B). In the adult spinal cord (C) the mVRF probes gave a neuronal staining pattern over the gray matter. Toluidine counterstaining showing that motoneurons in the ventral horn (D), interneurons in the deep part of the dorsal horn and around the central canal (not shown) where largely positive for mVRF mRNA. Scale bars = 0.1 mm (A), 0.1 mm (B), 0.25 mm (C), 0.015 mm (D).

**Figure 16** Effect of VEGF on embryonic day 8 (E8) chick sensory neurons as determined by % survival, % neurite outgrowth and average neurite length ( $\mu\text{m}$ ).

**Figure 17** Effects of VEGF and SOM175 on chick glia. Tested were CNS glial, peripheral glia and CNS oligodendrocytes.

**Figure 18** Effect of various SOM175 proteins on mouse astroglial cells. ■  $^3\text{H}$  (cpm)

- |    |     |  |
|----|-----|--|
|    | 1.  | FGF-2 (10 ng/ml) positive control      |
|    | 2.  | SOM $\Delta$ X6* 1 ng/ml               |
| 10 | 3.  | SOM $\Delta$ X6 10 ng/ml               |
|    | 4.  | SOM $\Delta$ X6 100 ng/ml              |
|    | 5.  | SOM $\Delta$ X6 1000 ng/ml             |
|    | 6.  | SOM $\Delta$ X6 1000 ng/ml, no heparin |
|    | 7.  | SOMX6** 1 ng/ml                        |
| 15 | 8.  | SOMX6 10 ng/ml                         |
|    | 9.  | SOMX6 100 ng/ml                        |
|    | 10. | SOMX6 1000 ng/ml                       |
|    | 11. | SOMX6 1000 ng/ml, no heparin           |

\* This refers to SOM175 absent exon 6;

20 \*\* This refers to SOM175.

**Figure 19** Effect of various SOM175 proteins on mouse oligodendroglial cells. ■  $^3\text{H}$  (cpm)

- |    |    |  |
|----|----|--|
|    | 1. | FGF-2 (10 ng/ml) positive control      |
| 25 | 2. | SOM $\Delta$ X6* 1 ng/ml               |
|    | 3. | SOM $\Delta$ X6 10 ng/ml               |
|    | 4. | SOM $\Delta$ X6 100 ng/ml              |
|    | 5. | SOM $\Delta$ X6 1000 ng/ml             |
|    | 6. | SOM $\Delta$ X6 1000 ng/ml, no heparin |
| 30 | 7. | SOMX6** 1 ng/ml                        |
|    | 8. | SOMX6 10 ng/ml                         |
|    | 9. | SOMX6 100 ng/ml                        |

10. SOMX6 1000 ng/ml

11. SOMX6 1000 ng/ml, no heparin

\* This refers to SOM175 absent exon 6;

\*\* This refers to SOM175.

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**Figure 20** Effect of various SOM175 proteins on mouse forebrain neurons. ■ % survival

1. FGF-2 (10 ng/ml) positive control

2. SOM $\Delta$ X6\* 1 ng/ml

10 3. SOM $\Delta$ X6 10 ng/ml

4. SOM $\Delta$ X6 100 ng/ml

5. SOM $\Delta$ X6 1000 ng/ml

6. SOM $\Delta$ X6 1000 ng/ml, no heparin

7. SOMX6\*\* 1 ng/ml

15 8. SOMX6 10 ng/ml

9. SOMX6 100 ng/ml

10. SOMX6 1000 ng/ml

11. SOMX6 1000 ng/ml, no heparin

\* This refers to SOM175 absent exon 6;

20 \*\* This refers to SOM175.

TABLE 1  
SUMMARY OF SEQUENCE IDENTITY NUMBERS

---

5	SEQ ID NO:1	Nucleotide sequence of VEGF <sub>165</sub>
	SEQ ID NO:2	Amino acid sequence of VEGF <sub>165</sub>
	SEQ ID NO:3	Nucleotide sequence of SOM175 (VEGF-like molecules)
	SEQ ID NO:4	Amino acid sequence of SOM175
10	SEQ ID NO:5	Nucleotide sequence of SOM175 absent exon 6
	SEQ ID NO:6	Amino acid sequence of SOM175 absent exon 6
	SEQ ID NO:7	Nucleotide sequence of SOM175 absent exon 6 and exon 7
	SEQ ID NO:8	Amino acid sequence of SOM175 absent exon 6 and exon 7
	SEQ ID NO:9	Nucleotide sequence of SOM175 absent exon 4
15	SEQ ID NO:10	Amino acid sequence of SOM175 absent exon 4
	SEQ ID NO:11	Oligonucleotide
	SEQ ID NO:12	Oligonucleotide
	SEQ ID NO:13	Oligonucleotide
	SEQ ID NO:14	Oligonucleotide

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20

### EXAMPLE 1

#### Human cDNA clones

The original SOM175 cDNA was isolated by screening a human foetal brain library (λzapII, Stratagene) with the cosmid D11S750 (Larsson *et al*, 1992). The plasmid was excised "*in vivo*" and a single 1.1kb cDNA was obtained. Three independent SOM175 cDNAs clones were also isolated from a human foetal spleen library (Stratagene, Uni-zap) using the above-mentioned SOM175 insert as a probe. Three clones were obtained: SOM175-4A, -5A and -6A. SOM175-5A is an alternately spliced clone with exon 4 being absent (SOM175-e4). These library screens were performed using hybridisation conditions recommended by the manufacturer of the library (Stratagene) and random primed insert of SOM175.



- 15 -

Two partial human SOM175 cDNAs have also isolated from a  $\lambda$ GT11 human melanoma cell line A2058 library (Clontech) cDNA library screens were performed using hybridisation conditions described by Church and Gilbert, 1984). In each case, the probe was generated by random priming of a PCR product derived from SOM175 (18f-  
5 700r).

### Mouse cDNA Clones

Human SOM175 was also used to screen a mouse neonatal whole brain cDNA library (Unizap, Stratagene). Four non-chimeric clones were isolated: M175-A, B, C, D. All  
10 clones were partial cDNAs and M175-C contained several introns. Three of these cDNAs lacked the exon 6.

Another clone referred to as M1 was completely sequenced and was found to contain the full open reading frame plus part of the 5'utr and total 3'utr.

15

## EXAMPLE 2

### DNA SEQUENCE ANALYSIS

The entire sequence of the cDNA clone (SOM175) was compiled and is shown in Figure 2 with its corresponding amino acid sequence. This sequence was screened for  
20 open reading frames using the MAP program (GCG, University of Wisconsin). A single open reading frame of 672bp was observed (see Figure 2). There appears to be little 5' untranslated sequences (2bp). The 3' untranslated region appears to be complete as it includes a poly-adenylation signal and poly-A tail.

25 Database homology searches were performed using the BLAST algorithm (run at NCBI, USA). This analysis revealed homology to several mammalian forms of VEGF (see Figure 3). The amount of homology between SOM175 and human VEGF<sub>165</sub> was determined using the BESTFIT program (GCG, University of Wisconsin; see Figures 4 and 5). Nucleotide homology was estimated at 69.7% and protein homology was  
30 estimated as at least 33.3% identity and 52.5% conservation using BESTFIT analysis. BLAST analysis on nucleotide sequences revealed the almost complete match to a human expressed sequence tag EST06302 (Adams *et al.*, 1993).

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These data indicate that SOM175 encodes a growth factor that has structural similarities to VEGF. Both genes show start and stop codons in similar positions and share discrete blocks of homology. All 8 cysteines as well as a number of other VEGF residues believed to be involved in dimerisation are conserved. These residues are Cysteine-47, 5 Proline-70, Cysteine-72, Valine-74, Arginine-77, Cysteine-78, Glycine-80, Cysteine-81, Cysteine-82, Cysteine-89, Proline-91, Cysteine-122 and Cysteine-124 and are shown in Figure 6. Given the structural conservation between VEGF and the SOM175 gene product it is also possible that they share functional similarities. It is proposed that SOM175 encodes a VEGF-like molecule that shares some properties with VEGF but has 10 unique properties of its own. The nucleotide sequence and corresponding amino acid sequence of VEGF<sub>165</sub> is shown in Figure 1.

### EXAMPLE 3

The percentage similarity and divergence between VEGF<sub>165</sub> family and SOM175 family 15 (protein) were analysed using the Clustal method, MegAlign Software, DNASTAR, Wisconsin. The results are shown in Tables 2.1 and 2.2. The alternatively spliced forms of SOM175 are abbreviated to SOM715-e6 where all of exon 6 is deleted; SOM715-e6 and 7 where all of exons 6 and 7 are deleted; and SOM175-e4 where all of exon 4 is deleted. The spliced form of SOM175 are shown in Figure 7. Genomic 20 maps of SOM175 showing intron/exon boundaries are shown in Figure 8a and 8b.

Table 2.1

A Percent nucleotide similarity between splice variants of SOM175 and  
 5 human VEGF<sub>165</sub>

	VEGF <sub>165</sub>	SOM175	SOM175-e6	SOM175-e6&7	SOM175-e4
VEGF <sub>165</sub>	***	34.9	39.7	41.4	37.0
10 SOM175		***	98.9	95.1	99.2
SOM175-e6			***	98.8	84.0
SOM175-e6&7				***	80.3
SOM175-e4					***

**B Percent nucleotide divergence between splice variants of SOM175 and human VEGF<sub>165</sub>**

5		VEGF <sub>165</sub>	SOM175	SOM175-e6	SOM175-e6&7	SOM175-e4
	VEGF <sub>165</sub>	***	41.7	41.6	41.7	41.8
	SOM175		***	0.2	0.2	0.0
	SOM175-e6			***	0.0	0.2
10	SOM175-e6&7				***	0.3
	SOM175-e4					***

**Table 2.2**

**15 A Percent amino acid identity between splice variants of SOM175 and human VEGF<sub>165</sub>**

		VEGF <sub>165</sub>	SOM175	SOM175-e6	SOM175-e6&7	SOM175-e4
20	VEGF <sub>165</sub>	***	31.4	42.3	33.5	40.6
	SOM175		***	74.7	73.7	99.1
	SOM175-e6			***	76.8	99.1
	SOM175-e6&7				***	99.1
	SOM175-e4					***

25

**B Percent amino acid divergence between splice variants of SOM175 and human VEGF<sub>165</sub>**

5		VEGF <sub>165</sub>	SOM175	SOM175-e6	SOM175-e6&7	SOM175-e4
	VEGF <sub>165</sub>	***	65.7	55.4	54.6	57.4
	SOM175		***	19.9	4.2	0.0
	SOM175-e6			***	0.0	0.0
10	SOM175-e6&7				***	0.0
	SOM175-e4					***

15

**EXAMPLE 4**

**BIOASSAYS TO DETERMINE THE FUNCTION OF SOM175**

Assays are conducted to evaluate whether SOM175 has similar activities to VEGF on endothelial cell function, angiogenesis and wound healing. Other assays are performed based on the results of receptor binding distribution studies.

20

**Assays of endothelial cell function**

*Endothelial cell proliferation.* Endothelial cell growth assays as described in Ferrara & Henzel (1989) and in Gospodarowicz *et al* (1989).

25 *Vascular permeability assay.* This assay, which utilises the Miles test in guinea pigs, will be performed as described in Miles & Miles (1952).

*Cell adhesion assay.* The influence of SOM175 on adhesion of polymorphs to endothelial cells is analysed.

30

*Chemotaxis.* This is performed using the standard Boyden chamber chemotaxis assay.

*Plasminogen activator assay.* Endothelial cells are tested for plasminogen activator and plasminogen activator inhibitor production upon addition of SOM175 (Pepper *et al* (1991)).

- 5 *Endothelial cell migration assay.* The ability of SOM175 to stimulate endothelial cells to migrate and form tubes is assayed as described in Montesano *et al* (1986).

#### **Angiogenesis Assay**

- SOM175 induction of an angiogenic response in chick chorioallantoic membrane is  
10 evaluated as described in Leung *et al* (1989).

Possible neurotrophic actions of SOM175 are assessed using the following assays:

#### **Neurite outgrowth assay and gene induction (PC12 cells)**

- 15 PC12 cells (a phaeochromocytoma cell line) respond to NGF and other neurotrophic factors by developing the characteristics of sympathetic neurons, including the induction of early and late genes and the extension of neurites. These cells are exposed to SOM175 and their response monitored (Drinkwater *et al* (1991); and Drinkwater *et al* (1993)).

20

#### **Cultured neurons from the Peripheral Nervous System (PNS)**

Primary cultures of the following PNS neurons are exposed to SOM175 and monitored for any response:

- sensory neurons from neural crest and dorsal root ganglia
- 25 - sympathetic neurons from sympathetic chain ganglia
- placode derived sensory neurons from nodose ganglia
- motoneurons from spinal cord

The assays are described in Suter *et al* (1992) and in Marinou *et al* (1992).

- 30 Where an *in vitro* response is observed, *in vivo* assays for properties such as uptake and retrograde transport are performed as described in Hendry *et al* (1992).

**Nerve regeneration (PNS)**

Where neurotrophic effects of SOM175 are observed, its possible role in the regeneration of axotomised sensory neurons, sympathetic neurons and motoneurons is analysed by the methods of Otto *et al* (1989); Yip *et al* (1984) and Hendry *et al* (1976).

**Actions of SOM175 on CNS neurons**

The ability of SOM175 to promote survival of central nervous system neurons is analysed as described in Hagg *et al* (1992); Williams *et al* (1986); Hefti (1986) and Kromer (1987).

**Wound Healing**

The ability of SOM175 to support wound healing are tested in the most clinically relevant model available, as described in Schilling *et al* (1959) and utilised by Hunt *et al* (1967).

**The Haemopoietic System**

A variety of *in vitro* and *in vivo* assays on specific cell populations of the haemopoietic system are available and are outlined below:

**20 Stem Cells****Murine**

A variety of novel *in vitro* murine stem cell assays have been developed using FACS-purified cells:

**25 (a) Repopulating Stem Cells**

These are cells capable of repopulating the bone marrow of lethally irradiated mice, and have the Lin<sup>-</sup>, Rh<sup>hi</sup>, Ly-6A/E<sup>+</sup>, c-kit<sup>+</sup> phenotype. The test substance is tested on these cells either alone, or by co-incubation with multiple factors, followed by measurement of cellular proliferation by <sup>3</sup>H thymidine incorporation.

**(b) Late Stage Stem Cells**

These are cells that have comparatively little bone marrow repopulating ability but can generate D13 CFU-S. These cells have the Lin<sup>-</sup>, Rh<sup>hi</sup>, Ly-6A/E<sup>+</sup>, c-kit<sup>+</sup> phenotype. The test substance is incubated with these cells for a period of time, injected into lethally irradiated recipients, and the number of D13 spleen colonies enumerated.

**(c) Progenitor-Enriched Cells**

These are cells that respond *in vitro* to single growth factors, and have the Lin<sup>-</sup>, Rh<sup>hi</sup>, Ly-6A/E<sup>+</sup>, c-kit<sup>+</sup> phenotype. This assay will show if SOM175 can act directly on haemopoietic progenitor cells. The test substance is incubated with these cells in agar cultures, and the number of colonies enumerated after 7-14 days.

**15 Atherosclerosis**

Smooth muscle cells play a crucial role in the development or initiation of atherosclerosis, requiring a change in their phenotype from a contractile to a synthetic state. Macrophages, endothelial cells, T lymphocytes and platelets all play a role in the development of atherosclerotic plaques by influencing the growth and phenotypic modulations of smooth muscle cell. An *in vitro* assay that measures the proliferative rate and phenotypic modulations of smooth muscle cells in a multicellular environment is used to assess the effect of SOM175 on smooth muscle cells. The system uses a modified Rose chamber in which different cell types are seeded onto opposite coverslips.

25

**Effects of SOM175 on bone**

The ability of SOM175 to regulate proliferation of osteoblasts is assayed as described in Lowe *et al* (1991). Any effects on bone resorption are assayed as described in Lowe *et al* (1991). Effects on osteoblast migration and changes in intracellular molecules (e.g. cAMP accumulation, alkaline phosphatase levels) are analysed as described in Midy *et al* (1994).

30



**Effects on skeletal muscle cells**

Effects of SOM175 on proliferation of myoblasts and development of myotubes can be determined as described by Ewton *et al* (1980) and by Gospodarowicz *et al* (1976).

5

**EXAMPLE 5****CLONING MURINE VEGF DNA****Isolation of cDNAs**

Murine VRF (mVRF) clones were selected from a lambda Zap new born whole brain cDNA library (Stratagene). Primary phage from high density filters ( $5 \times 10^4$  pfu/plate) were identified by hybridisation with a 682bp  $^{32}\text{P}$ -labelled probe generated by PCR from an hVRF cDNA (pSOM175) as described above. Hybridisation and stringent washes of nylon membranes (Hybond-N) were carried out at  $65^\circ\text{C}$  under conditions described by Church and Gilbert (1984). Positive plaques were picked, purified and excised *in vivo* to produce bacterial colonies containing cDNA clones in pBluescript SK<sup>-</sup>.

15

**Isolation of genomic clones**

Genomic clones were isolated from a mouse strain SV/129 library cloned in the lambda Fix II vector (Stratagene). High density filters ( $5 \times 10^4$  pfu/filter) were screened with a 563 bp  $^{32}\text{P}$ -labelled probe generated by PCR amplification of the nucleotide 233-798 region of the mVRF cDNA (see Figure 9). Positive clones were plugged and re-screened with filters containing 400-800 pfu. Large scale phage preparations were prepared using the QIAGEN lambda kit or by  $\text{ZnCl}_2$  purification (Santos, 1991).

25

**Nucleotide sequencing and analysis**

cDNAs were sequenced on both strands using a variety of vector-based and internal primers with Applied Biosystems Incorporated (ABI) dye terminator sequencing kits according to the manufacturer's specifications. Sequences were analysed on an ABI Model 373A automated DNA sequencer. Peptide homology alignments were performed using the program BESTFIT (GCG, Wisconsin).

30

### Identification of intron/exon boundaries

Identification of exon boundaries and flanking regions was carried out using PCR with mouse genomic DNA or mVRF genomic lambda clones as templates. The primers used in PCR to identify introns were derived from the hVRF sequence and to allow for potential human-mouse sequence mismatches annealing temperatures 5-10°C below the estimated  $T_m$  were used. All PCR products were sized by agarose gel electrophoresis and gel purified using QIAquick spin columns (Qiagen) and the intron/exon boundaries were sequenced directly from these products. In addition, some splice junctions were sequenced from subcloned genomic fragments of MVRF.

10 Intron/exon boundaries were identified by comparing cDNA and genomic DNA sequences.

### Northern analysis

Total cellular RNA was prepared from a panel of fresh normal adult mouse tissues (brain, kidney, liver, muscle) using the method of Chomczynski and Sacchi (1987). 20µg of total RNA were electrophoresed, transferred to a nylon membrane (Hybond N, Amersham) and hybridised under standard conditions (Church & Gilbert, 1984). Filters were washed at 65°C in 0.1xSSC (20xSSC is 3M NaCl/0.3M trisodium citrate), 0.1% SDS and exposed to X-ray film with intensifying screens at -70°C for 1-3 days.

### Characterisation of mVRF cDNAs

Murine VRF homologues were isolated by screening a murine cDNA library with an hVRF cDNA clone. Five clones of sizes varying from 0.8-1.5 kb were recovered and sequenced. The cDNA sequences were compiled to give a full length 1041 bp cDNA sequence covering the entire open reading frame (621 bp or 564 bp depending on the splice form, see below) and 3' UTR (379 bp), as well as 163 bp of the 5' UTR (Figure 9).

30 The predicted initiation codon matched the position of the start codon in hVRF. One other out of frame ATG was located at position -47 and two termination codons were observed upstream (positions -9 and -33, respectively) and in-frame with the putative

initiation codon.

The predicted N-terminal signal peptide of hVRF appears to be present in mVRF with 81% identity (17/21 amino acids). Peptide cleavage within mVRF is expected to occur after residue 21 (Figure 10). These data suggest that mature mVRF is secreted and could therefore conceivably function as a growth factor.

As with hVRF, two open reading frames (ORFs) were detected in cDNAs isolated by library screening. Four of five clones were found to be alternatively spliced and lacked a 101 bp fragment homologous to exon 6 of hVRF. The predicted peptide sequences of the two isoforms of mVRF were determined and aligned with the corresponding human isoforms (Figure 10).

The message encoding mVRF<sub>186</sub> contains a 621 bp ORF with coding sequences terminating at position +622, towards the end of exon 7 (Figure 9). The smaller message encoding mVRF<sub>167</sub> actually terminates downstream of the +622 TAG site due to a frame shift resulting from splicing out of the 101 bp exon 6 and the introduction of a stop codon (TGA) at position +666, near the beginning of exon 8 (Figure 9).

The mVRF<sub>186</sub> protein has strong homology to the amino and central portions of VEGF while the carboxyl end is completely divergent and is alanine rich. mVRF<sub>167</sub> possesses these similarities and also maintains homology to mVEGF right through to the C-terminus (Figure 11). The overall homology of mVRF<sub>167</sub> to hVRF<sub>167</sub> was 85% identity and 92% similarity, respectively (Figure 10). Likewise, homology between mVRF<sub>167</sub> and mVEGF (Breier *et al*, 1992) was 49% identity and 71% conservative amino acid substitution, respectively (Figure 11).

A canonical vertebrate polyadenylation signal (AATAAA) (Birnstiel *et al*, 1986) was not present in the mVRF cDNA, however, the closely matching sequence GATAAA is present at similar positions in both mouse and human VRF cDNAs (Figure 9). In contrast to hVRF, mVRF was found to contain an AC dinucleotide repeat at the

extreme 3' end of the 3' UTR (nucleotide positions 998 to 1011, Figure 9).

Polymorphism of this repeat region was observed between some of the mVRF cDNAs, with the number of dinucleotides varying from 7 to 11.

## 5 Genomic characterisation of mVRF

Intron/exon boundaries (Table 3) were mapped using primers which flanked sequences homologous to the corresponding hVRF boundaries. Introns I, III, IV and VI of mVRF (Table 3, Figure 12) were smaller than the hVRF intervening sequences. The complete genomic sequence was compiled from the 5' UTR of mVRF through to intron VI, the largest intervening region (2.2 kb), by sequencing amplified introns and cloned genomic portions of mVRF. There was only one major difference in genomic structure between mVRF and hVRF and that was the exon 7/intron VI boundary of mVRF was located 10bp further downstream in relation to the cDNA sequence, hence exon 7 in mVRF is 10bp longer than the corresponding exon in hVRF.

Exons 6 and 7 are contiguous in mVRF, as has been found to occur in the human homologue. The strong sequence homology between exon 6 of mVRF and hVRF (Figure 10) suggests that this sequence is not a retained intronic sequence but rather encodes a functional part of the VRF<sub>186</sub> isoform.

General intron/exon structure is conserved between the various members of the VEGF gene family (VEGF, PIGF, hVRF) and therefore it is not surprising that the overall genomic organisation of the mVRF gene is very similar to these genes (Figure 12).

Previous comparative mapping studies have shown that the region surrounding the human multiple endocrine neoplasia type 1 disease locus on chromosome 11q13 is syntenic with the proximal segment of mouse chromosome 19 (Rochelle *et al*, 1992). Since the inventors have mapped the hVRF gene to within 1kb of the human *MEN1* locus (see above) it is most likely that the murine VRF gene maps near the centromere of chromosome 19.

**Expression studies of mVRF**

Northern analysis of RNA from adult mouse tissues (muscle, heart, lung and liver) showed that expression appears to be ubiquitous and occurs primarily as a major band of approximately 1.3kb in size (Figure 14). This is somewhat different to the pattern observed for hVRF in which two major bands of 2.0 and 5.5 kb have been identified in all tissues examined. The 1.3 kb murine message presumably corresponds to the shorter of the human transcripts and the size variation thereof is most likely due to a difference in the length of the respective 5' UTRs.

10

**EXAMPLE 6****EXPRESSION OF MURINE VEGF IN PRE- AND POST-NATAL MOUSE****Animals**

Timed pregnant (n=4) and young adult (n=2) mice (C57 inbred strain, ALAB, Sweden) were sacrificed with carbon dioxide, and the relevant tissues were taken out and frozen on a chuck. Tissues were kept at -70°C until further use. Two gestational ages was used in this study; embryonic day 8 (E8), 14 and E17.

***In situ* hybridisation histochemistry**

*In situ* hybridisation was performed as previously described (Dagerlind *et al*, 1992). Briefly, transverse sections (14µm) were cut in a cryostat (Microm, Germany), thawed onto Probe-On slides (Fisher Scientific, USA) and stored in black sealed boxes at -70°C until used. The sequences of the synthetic 42-mer oligonucleotides complementary to mRNA encoding mVRF were ACCACCACCTCCCTGGGCTGGCATGTGGCACGTGCATAAACG [SEQ ID NO:11] (complementary to nt 120-161) and AGTTGTTTGACCACATTGCCCATGAGTTCCATGCTCAGAGGC [SEQ ID NO:12] (complementary to nt 162-203). To detect the two alternative splice forms oligonucleotide GATCCTGGGGCTGGAGTGGGATGGATGATGTCAGCTGG [SEQ ID NO:13] (complementary to nt xxx-xxx) and GCGGGCAGAGGATCCTGGGGCTGTCTGGCCTCACAGCACT [SEQ ID NO:14] were used. The probes were labeled at the 3'-end with deoxyadenosine-alpha[thio]triphosphate [<sup>35</sup>S] (NEN, USA) using terminal deoxynucleotidyl

transferase (IBI, USA) to a specific activity of  $7-10 \times 10^8$  cpm/ $\mu$ g and hybridised to the sections without pretreatment for 16-18 h at 42°C. The hybridisation mixture contained: 50% v/v formamide, 4 x SSC (1 x SSC = 0.15M NaCl and 0.015M sodium-citrate), 1 x Denhardt's solution (0.02% each of polyvinyl-pyrrolidone, BSA and Ficoll), 1% v/v sarcosyl (N-lauroylsarcosine; Sigma), 0.02M phosphate buffer (pH 7.0), 10% w/v dextran sulfate (Pharmacia, Sweden), 250 $\mu$ g/ml yeast tRNA (Sigma), 500 $\mu$ g/ml sheared and heat denatured salmon sperm DNA (Sigma) and 200mM dithiothreitol (DTT; LKB, Sweden). In control sections, the specificity of both probes was checked by adding a 20-fold excess of unlabeled probe to the hybridisation mixture. In addition, adjacent sections were hybridised with a probe unrelated to this study which gave a different expression pattern. Following hybridisation the sections were washed several times in 1 x SSC at 55°C, dehydrated in ethanol and dipped in NTB2 nuclear track emulsion (Kodak, USA). After 3-5 weeks the sections were development in D-19 developer (Kodak, USA) and cover-slipped. In some cases, sections were exposed to an autoradiographic film (Beta-max autoradiography film Amersham Ltd, UK) prior to emulsion-dipping.

The four different probes gave identical hybridisation patterns in all tissues examined. Mouse VRF expression was detected already in the E8 embryo, in which positive signal was recorded over structures most likely corresponding to the neuronal tube. In sagittal sections of E14 mouse embryo the strongest hybridisation signal was present over heart and in the nervous system, especially cerebral cortex (Figure 14A). A low level of expression was present in all other tissues. At a later gestational age, E17, a high mVRF mRNA signal was confined to the heart and brown fat tissue in the back and around the neck (Figure 14B). Clearly positive hybridisation signals were present in the gray of the spinal cord and in the tongue (Figure 14B). Expression in the cerebral cortex was clearly reduced compared to day 14. The weak background expression seen in the E14 embryo in for example muscle, had decreased at this gestational age. A strong mVRF mRNA hybridisation signal was present solely over the heart and in the brown fat in the young adult mice (Figure 14C). The signal over the heart was evenly distributed over the entire ventricular wall, including the papillary muscles (Figure 14D). In sections of heart tissue hybridised with an

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excess of cold probe, no specific labeling over background signal was recorded (Figure 14E).

Apart from the heart, mVRF mRNA signal was present over certain tissues on the outside of the thoracic cage that morphologically resembled brown fat. This was verified with sudan black counterstaining, which showed a strong staining in the same areas (Figure 15A and 15B). In transverse sections of adult mouse spinal cord, the mVRF probes gave a neuronal staining pattern over the gray matter (Figure 15C). Counterstaining with toluidine (Figure 15D) showed that motoneurons in the ventral horn (Figure 15C and 15D), interneurons (Figure 15C) in the deep part of the dorsal horn and around the central canal where to a large extent positive for mVRF mRNA.

#### EXAMPLE 7

##### EFFECTS OF VEGF AND SOM175 PROTEINS ON CHICK

##### SENSORY NEURONS

The effects of VEGF and SOM175 proteins on embryonic day 8 chick sensory neurons were determined using the method of Nurcombe *et al* (1992). The neuronal assay was read at 48 hours using 2000 cells per assay well. The results were obtained using <sup>3</sup>H-thymidine counts. The percentage survival of neurons, neurite outgrowth and average neurite length in  $\mu\text{m}$  were determined using NGF as positive control and various concentrations of VEGF, VEGF in the presence of heparin and VEGF in the presence of heparin and 5  $\mu\text{M}$ , 5'-flurouracil (5FU). 5FU kills glial cells.

The results are shown in Figure 16. The results show that VEGF is effective in promoting neuronal survival but that this requires the presence of glial cells. Figure 17 shows the results of the effect of VEGF and SOM175 on three types of chick glia. The glia tested were CNS glia, peripheral glia and CNS oligodendrocytes. Heparin was used as 10  $\mu\text{g/ml}$  in all cultures and the assay was read at 24 hours. Results were measured in <sup>3</sup>H-thymidine counts using 2000 cells per well.

The results show that for chick central and peripheral neurons, astroglia were markedly stimulated to proliferate by SOM175 in the presence of heparin but that chick oligodendrocytes showed negligible increase in the rate of division.

5

### EXAMPLE 8

#### EFFECTS OF SOM175 PROTEINS ON MOUSE PRIMARY AND CENTRAL NEURONS

The results in Example 7 show that VEGF isoform had an effect on chick primary  
10 and central neurons through the agency of the astroglial cells. Similar experiments were repeated in mouse cells.

#### Culture conditions

Neuronal and glial cells for all *in vitro* experiments were prepared and cultured  
15 according to the techniques described in "Methods in Neurosciences (Vol. 2): Cell Culture" Ed. P.M. Conn, Academic Press, San Diego, 1990, pp33-46 for astroglial cells, pp56-74 for oligodendroglial cells, and pp87-102 for central neurons.

Cells were plated onto 24-well culture clusters (Nunc) coated with poly-L-ornithine  
20 (0.1 mg/ml, 1h) at a density of 2,000 cells/well. After 48 hours in culture, neurons were counted in the wells under inverted phase light using well established techniques (Maruta *et al.* 1993) and glial cells assessed with [<sup>3</sup>H]thymidine uptake to monitor cell division rates as below. Heparin (10µg/ml, low molecular weight fraction, Sigma Chemical Corp.) was present at all times in the culture media except  
25 where noted. The neuronal cultures were supplemented with 5mM 5-fluoro-2-deoxyuridine (Sigma) to suppress background glial growth.

#### <sup>3</sup>H-Thymidine incorporation assay for glial cell proliferation

The cells were pulsed for 14h with <sup>3</sup>H-thymidine (specific activity 103 µCi/ug) from  
30 a stock concentration of 0.1 mCi/ml in standard medium, giving a final incubating volume of 20 µl/well. The contents of the wells were harvested and absorbed onto nitrocellulose paper (Titertek, Flow). Remaining adherent cells were removed by



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incubation with trypsin/versene (CSL Limited, Victoria, Australia) for 5 min. This procedure was carried out twice. The nitrocellulose discs were washed in a standard Titertek harvester (Flow) using first distilled water, and then methanol. The nitrocellulose discs were dried, scintillation fluid (containing 5% v/v Triton-X) added  
5 and the discs counted on a scintillation counter.

Greatest activity was seen with preparations of SOM175 absent exon 6 (SOM $\Delta$ X6) on mouse astroglial cell cultures, where there was a significant stimulus to their proliferation when delivered in conjunction with heparin (Figure 16). Little stimulus  
10 was given to the proliferation of oligodendroglial cells (Figure 17), and very little discernable potentiation of the survival response of isolated forebrain neurons (Figure 18). The standard deviation on all three graphs for each point was less than 8%.

The viability of neurons can be maintained by promoting glial cell proliferation.  
15 Furthermore, SOM $\Delta$ X6 is a good inducer of astroglial proliferation and may be expressed in conjunction with the formation of astroglial endfeet on central nervous system endothelial cells.

Those skilled in the art will appreciate that the invention described herein is  
20 susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

25

**TABLE 3**  
**Splice junctions of the murine VRF gene**

5	5' UTR* .....	Exon 1 >223bp	CCCAGgtacgtgcgt	Intron I	495bp
	ttccccacagGCCCC	Exon 2 43bp	GAAAGgtaataatag	Intron II	288bp
	ctgcccacagTGGTG	Exon 3 197bp	TGCAGgtaccagggc	Intron III	196bp
	ctgagcacagATCCT	Exon 4 74bp	TGCAGgtgccagccc	Intron IV	182bp
	ctcttttcagACCTA	Exon 5 36bp	GACAGattcttggtg	Intron V	191bp
10	ctcctcctagGGTTG	Exon 6 101bp		(no intron)	
	CCCACTCCAGCCCCA	Exon 7 135bp	TGTAGgtaaggagtc	Intron VI	~2200bp
	cactccccagGTGCC	Exon 8 394bp	AGAGATGGAGACACT		

Uppercase and lowercase letters denote exonic and intronic sequences respectively.

15 \* Indicates that the 5' end of exon 1 has not yet been determined.

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## SEQUENCE LISTING

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GENETIC SEQUENCE ENCODING SAME

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(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

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## (2) INFORMATION FOR SEQ ID NO:1:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 649 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA

## (ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 17...589

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CTT GCC TTG CTG CTC TAC CTC CAC CAT GCC AAG TGG TCC CAG GCT GCA	97
Leu Ala Leu Leu Leu Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala	
15 20 25	
CCC ATG GCA GAA GGA GGA GGG CAG AAT CAT CAC GAA GTG GTG AAG TTC	145
Pro Met Ala Glu Gly Gly Gly Gln Asn His His Glu Val Val Lys Phe	
30 35 40	
ATG GAT GTC TAT CAG CGC AGC TAC TGC CAT CCA ATC GAG ACC CTG GTG	193
Met Asp Val Tyr Gln Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val	
45 50 55	
GAC ATC TTC CAG GAG TAC CCT GAT GAG ATC GAG TAC ATC TTC AAG CCA	241
Asp Ile Phe Gln Glu Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro	
60 65 70 75	
TCC TGT GTG CCC CTG ATG CGA TGC GGG GGC TGC TGC AAT GAC GAG GGC	289
Ser Cys Val Pro Leu Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly	
80 85 90	

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CTG GAG TGT GTG CCC ACT GAG GAG TCC AAC ATC ACC ATG CAG ATT ATG	337
Leu Glu Cys Val Pro Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met	
95 100 105	
CGG ATC AAA CCT CAC CAA GGC CAG CAC ATA GGA GAG ATG AGC TTC CTA	385
Arg Ile Lys Pro His Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu	
110 115 120	
CAG CAC AAC AAA TGT GAA TGC AGA CCA AAG AAA GAT AGA GCA AGA CAA	433
Gln His Asn Lys Cys Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln	
125 130 135	
GAA AAT CCC TGT GGG CCT TGC TCA GAG CGG AGA AAG CAT TTG TTT GTA	481
Glu Asn Pro Cys Gly Pro Cys Ser Glu Arg Arg Lys His Leu Phe Val	
140 145 150 155	
CAA GAT CCG CAG ACG TGT AAA TGT TCC TGC AAA AAC ACA GAC TCG CGT	529
Gln Asp Pro Gln Thr Cys Lys Cys Ser Cys Lys Asn Thr Asp Ser Arg	
160 165 170	
TGC AAG GCG AGG CAG CTT GAG TTA AAC GAA CGT ACT TGC AGA TGT GAC	577
Cys Lys Ala Arg Gln Leu Glu Leu Asn Glu Arg Thr Cys Arg Cys Asp	
175 180 185	
AAG CCG AGG CGG TGAGCCGGGC AGGAGGAAGG AGCCTCCCTC AGCGTTTCGG	629
Lys Pro Arg Arg	
190	
GAACCAGATC TCTCACCAGG	649

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- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Met Ala Glu Gly	
20 25 30	
Gly Gly Gln Asn His His Glu Val Val Lys Phe Met Asp Val Tyr Gln	
35 40 45	
Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu	
50 55 60	



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Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser Cys Val Pro Leu  
 65 70 75 80  
 Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu Glu Cys Val Pro  
 85 90 95  
 Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His  
 100 105 110  
 Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln His Asn Lys Cys  
 115 120 125  
 Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu Asn Pro Cys Gly  
 130 135 140  
 Pro Cys Ser Glu Arg Arg Lys His Leu Phe Val Gln Asp Pro Gln Thr  
 145 150 155 160  
 Cys Lys Cys Ser Cys Lys Asn Thr Asp Ser Arg Cys Lys Ala Arg Gln  
 165 170 175  
 Leu Glu Leu Asn Glu Arg Thr Cys Arg Cys Asp Lys Pro Arg Arg  
 180 185 190

## (2) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1094 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 3..624

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CC ATG AGC CCT CTG CTC CGC CGC CTG CTG CTC GCC GCA CTC CTG CAG 47  
 Met Ser Pro Leu Leu Arg Arg Leu Leu Leu Ala Ala Leu Leu Gln  
 1 5 10 15  
 CTG GCC CCC GCC CAG GCC CCT GTC TCC CAG CCT GAT GCC CCT GGC CAC 95  
 Leu Ala Pro Ala Gln Ala Pro Val Ser Gln Pro Asp Ala Pro Gly His  
 20 25 30  
 CAG AGG AAA GTG GTG TCA TGG ATA GAT GTG TAT ACT CGC GCT ACC TGC 143  
 Gln Arg Lys Val Val Ser Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys  
 35 40 45

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CAG CCC CGG GAG GTG GTG GTG CCC TTG ACT GTG GAG CTC ATG GGC ACC Gln Pro Arg Glu Val Val Val Pro Leu Thr Val Glu Leu Met Gly Thr 50 55 60	191
GTG GCC AAA CAG CTG GTG CCC AGC TGC GTG ACT GTG CAG CGC TGT GGT Val Ala Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly 65 70 75	239
GGC TGC TGC CCT GAC GAT GGC CTG GAG TGT GTG CCC ACT GGG CAG CAC Gly Cys Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr Gly Gln His 80 85 90 95	287
CAA GTC CGG ATG CAG ATC CTC ATG ATC CGG TAC CCG AGC AGT CAG CTG Gln Val Arg Met Gln Ile Leu Met Ile Arg Tyr Pro Ser Ser Gln Leu 100 105 110	335
GGG GAG ATG TCC CTG GAA GAA CAC AGC CAG TGT GAA TGC AGA CCT AAA Gly Glu Met Ser Leu Glu Glu His Ser Gln Cys Glu Cys Arg Pro Lys 115 120 125	383
AAA AAG GAC AGT GCT GTG AAG CCA GAC AGG GCT GCC ACT CCC CAC CAC Lys Lys Asp Ser Ala Val Lys Pro Asp Arg Ala Ala Thr Pro His His 130 135 140	431
CGT CCC CAG CCC CGT TCT GTT CCG GGC TGG GAC TCT GCC CCC GGA GCA Arg Pro Gln Pro Arg Ser Val Pro Gly Trp Asp Ser Ala Pro Gly Ala 145 150 155	479
CCC TCC CCA GCT GAC ATC ACC CAT CCC ACT CCA GCC CCA GGC CCC TCT Pro Ser Pro Ala Asp Ile Thr His Pro Thr Pro Ala Pro Gly Pro Ser 160 165 170 175	527
GCC CAC GCT GCA CCC AGC ACC ACC AGC GCC CTG ACC CCC GGA CCT GCC Ala His Ala Ala Pro Ser Thr Thr Ser Ala Leu Thr Pro Gly Pro Ala 180 185 190	575
GCT GCC GCT GCC GAC GCC GCA GCT TCC TCC GTT GCC AAG GGC GGG GCT T Ala Ala Ala Ala Asp Ala Ala Ala Ser Ser Val Ala Lys Gly Gly Ala 195 200 205	624
AGAGCTCAAC CCAGACACCT GCAGGTGCCG GAAGCTGCGA AGGTGACACA TGGCTTTTCA	684
GACTCAGCAG GGTGACTTGC CTCAGAGGCT ATATCCCAGT GGGGGAACAA AGGGGAGCCT	744
GGTAAAAAAC AGCCAAGCCC CCAAGACCTC AGCCCAGGCA GAAGCTGCTC TAGGACCTGG	804
GCCTCTCAGA GGGCTCTTCT GCCATCCCTT GTCTCCCTGA GGCCATCATC AAACAGGACA	864
GAGTTGGAAG AGGAGACTGG GAGGCAGCAA GAGGGGTCAC ATACCAGCTC AGGGGAGAAT	924
GGAGTACTGT CTCAGTTTCT AACCACCTCTG TGCAAGTAAG CATCTTACAA CTGGCTCTTC	984
CTCCCTCAC TAAGAAGACC CAAACCTCTG CATAATGGGA TTTGGGCTTT GGTACAAGAA	1044
CTGTGACCCC CAACCCTGAT AAAAGAGATG GAAGGAAAAA AAAAAAAAAA	1094

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## (2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 207 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```

Met Ser Pro Leu Leu Arg Arg Leu Leu Leu Ala Ala Leu Leu Gln Leu
 1             5             10             15

Ala Pro Ala Gln Ala Pro Val Ser Gln Pro Asp Ala Pro Gly His Gln
      20             25             30

Arg Lys Val Val Ser Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys Gln
      35             40             45

Pro Arg Glu Val Val Val Pro Leu Thr Val Glu Leu Met Gly Thr Val
      50             55             60

Ala Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly Gly
      65             70             75             80

Cys Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr Gly Gln His Gln
      85             90             95

Val Arg Met Gln Ile Leu Met Ile Arg Tyr Pro Ser Ser Gln Leu Gly
      100            105            110

Glu Met Ser Leu Glu Glu His Ser Gln Cys Glu Cys Arg Pro Lys Lys
      115            120            125

Lys Asp Ser Ala Val Lys Pro Asp Arg Ala Ala Thr Pro His His Arg
      130            135            140

Pro Gln Pro Arg Ser Val Pro Gly Trp Asp Ser Ala Pro Gly Ala Pro
      145            150            155            160

Ser Pro Ala Asp Ile Thr His Pro Thr Pro Ala Pro Gly Pro Ser Ala
      165            170            175

His Ala Ala Pro Ser Thr Thr Ser Ala Leu Thr Pro Gly Pro Ala Ala
      180            185            190

Ala Ala Ala Asp Ala Ala Ala Ser Ser Val Ala Lys Gly Gly Ala
      195            200            205

```

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## (2) INFORMATION FOR SEQ ID NO:5:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 993 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 3..566

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```

CC ATG AGC CCT CTG CTC CGC CGC CTG CTG CTC GCC GCA CTC CTG CAG      47
  Met Ser Pro Leu Leu Arg Arg Leu Leu Leu Ala Ala Leu Leu Gln
    1                   5                   10                   15

CTG GCC CCC GCC CAG GCC CCT GTC TCC CAG CCT GAT GCC CCT GGC CAC      95
  Leu Ala Pro Ala Gln Ala Pro Val Ser Gln Pro Asp Ala Pro Gly His
                   20                   25                   30

CAG AGG AAA GTG GTG TCA TGG ATA GAT GTG TAT ACT CGC GCT ACC TGC      143
  Gln Arg Lys Val Val Ser Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys
                   35                   40                   45

CAG CCC CGG GAG GTG GTG GTG CCC TTG ACT GTG GAG CTC ATG GGC ACC      191
  Gln Pro Arg Glu Val Val Val Pro Leu Thr Val Glu Leu Met Gly Thr
                   50                   55                   60

GTG GCC AAA CAG CTG GTG CCC AGC TGC GTG ACT GTG CAG CGC TGT GGT      239
  Val Ala Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly
                   65                   70                   75

GGC TGC TGC CCT GAC GAT GGC CTG GAG TGT GTG CCC ACT GGG CAG CAC      287
  Gly Cys Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr Gly Gln His
                   80                   85                   90                   95

CAA GTC CGG ATG CAG ATC CTC ATG ATC CGG TAC CCG AGC AGT CAG CTG      335
  Gln Val Arg Met Gln Ile Leu Met Ile Arg Tyr Pro Ser Ser Gln Leu
                   100                  105                  110

GGG GAG ATG TCC CTG GAA GAA CAC AGC CAG TGT GAA TGC AGA CCT AAA      383
  Gly Glu Met Ser Leu Glu Glu His Ser Gln Cys Glu Cys Arg Pro Lys
                   115                  120                  125

AAA AAG GAC AGT GCT GTG AAG CCA GAT AGC CCC AGG CCC CTC TGC CCA      431
  Lys Lys Asp Ser Ala Val Lys Pro Asp Ser Pro Arg Pro Leu Cys Pro
                   130                  135                  140

```

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CGC TGC ACC CAG CAC CAC CAG CGC CCT GAC CCC CGG ACC TGC CGC TGC 479  
 Arg Cys Thr Gln His His Gln Arg Pro Asp Pro Arg Thr Cys Arg Cys  
 145 150 155

CGC TGC CGA CGC CGC AGC TTC CTC CGT TGC CAA GGG CGG GGC TTA GAG 527  
 Arg Cys Arg Arg Arg Ser Phe Leu Arg Cys Gln Gly Arg Gly Leu Glu  
 160 165 170 175

CTC AAC CCA GAC ACC TGC AGG TGC CGG AAG CTG CGA AGG TGACACATGG 576  
 Leu Asn Pro Asp Thr Cys Arg Cys Arg Lys Leu Arg Arg  
 180 185

CTTTTCAGAC TCAGCAGGGT GACTTGCCTC AGAGGCTATA TCCCAGTGGG GGAACAAAGG 636

GGAGCCTGGT AAAAAACAGC CAAGCCCCCA AGACCTCAGC CCAGGCAGAA GCTGCTCTAG 696

GACCTGGGCC TCTCAGAGGG CTCTTCTGCC ATCCCTTGTC TCCCTGAGGC CATCATCAAA 756

CAGGACAGAG TTGGAAGAGG AGACTGGGAG GCAGCAAGAG GGGTCACATA CCAGCTCAGG 816

GGAGAATGGA GTACTGTCTC AGTTTCTAAC CACTCTGTGC AAGTAAGCAT CTTACAACTG 876

GCTCTTCCTC CCCTCACTAA GAAGACCCAA ACCTCTGCAT AATGGGATTT GGGCTTTGGT 936

ACAAGAACTG TGACCCCCAA CCCTGATAAA AGAGATGGAA GGAAAAAAAA AAAAAAA 993

## (2) INFORMATION FOR SEQ ID NO:6:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 188 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Ser Pro Leu Leu Arg Arg Leu Leu Leu Ala Ala Leu Leu Gln Leu  
 1 5 10 15

Ala Pro Ala Gln Ala Pro Val Ser Gln Pro Asp Ala Pro Gly His Gln  
 20 25 30

Arg Lys Val Val Ser Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys Gln  
 35 40 45

Pro Arg Glu Val Val Val Pro Leu Thr Val Glu Leu Met Gly Thr Val  
 50 55 60

Ala Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly Gly  
 65 70 75 80

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Cys	Cys	Pro	Asp	Asp	Gly	Leu	Glu	Cys	Val	Pro	Thr	Gly	Gln	His	Gln		
				85					90					95			
Val	Arg	Met	Gln	Ile	Leu	Met	Ile	Arg	Tyr	Pro	Ser	Ser	Gln	Leu	Gly		
				100					105					110			
Glu	Met	Ser	Leu	Glu	Glu	His	Ser	Gln	Cys	Glu	Cys	Arg	Pro	Lys	Lys		
				115					120					125			
Lys	Asp	Ser	Ala	Val	Lys	Pro	Asp	Ser	Pro	Arg	Pro	Leu	Cys	Pro	Arg		
				130					135					140			
Cys	Thr	Gln	His	His	Gln	Arg	Pro	Asp	Pro	Arg	Thr	Cys	Arg	Cys	Arg		
145					150					155					160		
Cys	Arg	Arg	Arg	Ser	Phe	Leu	Arg	Cys	Gln	Gly	Arg	Gly	Leu	Glu	Leu		
				165					170					175			
Asn	Pro	Asp	Thr	Cys	Arg	Cys	Arg	Lys	Leu	Arg	Arg						
				180					185								

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 858 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA

(ix) **FEATURE:**

- (A) NAME/KEY: CDS  
(B) LOCATION: 3..431

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CC ATG AGC CCT CTG CTC CGC CGC CTG CTG CTC GCC GCA CTC CTG CAG	47
Met Ser Pro Leu Leu Arg Arg Leu Leu Leu Ala Ala Leu Leu Gln	
1 5 10 15	
CTG GCC CCC GCC CAG GCC CCT GTC TCC CAG CCT GAT GCC CCT GGC CAC	95
Leu Ala Pro Ala Gln Ala Pro Val Ser Gln Pro Asp Ala Pro Gly His	
20 25 30	
CAG AGG AAA GTG GTG TCA TGG ATA GAT GTG TAT ACT CGC GCT ACC TGC	143
Gln Arg Lys Val Val Ser Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys	
35 40 45	
CAG CCC CGG GAG GTG GTG GTG CCC TTG ACT GTG GAG CTC ATG GGC ACC	191
Gln Pro Arg Glu Val Val Val Pro Leu Thr Val Glu Leu Met Gly Thr	
50 55 60	

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GTG GCC AAA CAG CTG GTG CCC AGC TGC GTG ACT GTG CAG CGC TGT GGT	239
Val Ala Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly	
65 70 75	
GGC TGC TGC CCT GAC GAT GGC CTG GAG TGT GTG CCC ACT GGG CAG CAC	287
Gly Cys Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr Gly Gln His	
80 85 90 95	
CAA GTC CGG ATG CAG ATC CTC ATG ATC CGG TAC CCG AGC AGT CAG CTG	335
Gln Val Arg Met Gln Ile Leu Met Ile Arg Tyr Pro Ser Ser Gln Leu	
100 105 110	
GGG GAG ATG TCC CTG GAA GAA CAC AGC CAG TGT GAA TGC AGA CCT AAA	383
Gly Glu Met Ser Leu Glu Glu His Ser Gln Cys Glu Cys Arg Pro Lys	
115 120 125	
AAA AAG GAC AGT GCT GTG AAG CCA GAT AGG TGC CGG AAG CTG CGA AGG	431
Lys Lys Asp Ser Ala Val Lys Pro Asp Arg Cys Arg Lys Leu Arg Arg	
130 135 140	
TGACACATGG CTTTTCAGAC TCAGCAGGGT GACTTGCCTC AGAGGCTATA TCCCAGTGGG	491
GGAACAAAGG GGAGCCTGGT AAAAAACAGC CAAGCCCCCA AGACCTCAGC CCAGGCAGAA	551
GCTGCTCTAG GACCTGGGCC TCTCAGAGGG CTCTTCTGCC ATCCCTTGTC TCCCTGAGGC	611
CATCATCAAA CAGGACAGAG TTGGAAGAGG AGACTGGGAG GCAGCAAGAG GGGTCACATA	671
CCAGCTCAGG GGAGAATGGA GTACTGTCTC AGTTTCTAAC CACTCTGTGC AAGTAAGCAT	731
CTTACAACCTG GCTCTTCCTC CCCTCACTAA GAAGACCCAA ACCTCTGCAT AATGGGATTT	791
GGGCTTTGGT ACAAGAACTG TGACCCCCAA CCCTGATAAA AGAGATGGAA GGAAAAAAAA	851
AAAAAAAA	858

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## (2) INFORMATION FOR SEQ ID NO:8:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 143 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met	Ser	Pro	Leu	Leu	Arg	Arg	Leu	Leu	Leu	Ala	Ala	Leu	Leu	Gln	Leu	
1				5					10					15		
Ala	Pro	Ala	Gln	Ala	Pro	Val	Ser	Gln	Pro	Asp	Ala	Pro	Gly	His	Gln	
			20					25					30			
Arg	Lys	Val	Val	Ser	Trp	Ile	Asp	Val	Tyr	Thr	Arg	Ala	Thr	Cys	Gln	
		35					40					45				
Pro	Arg	Glu	Val	Val	Val	Pro	Leu	Thr	Val	Glu	Leu	Met	Gly	Thr	Val	
		50					55				60					
Ala	Lys	Gln	Leu	Val	Pro	Ser	Cys	Val	Thr	Val	Gln	Arg	Cys	Gly	Gly	
65					70					75					80	
Cys	Cys	Pro	Asp	Asp	Gly	Leu	Glu	Cys	Val	Pro	Thr	Gly	Gln	His	Gln	
				85					90					95		
Val	Arg	Met	Gln	Ile	Leu	Met	Ile	Arg	Tyr	Pro	Ser	Ser	Gln	Leu	Gly	
		100						105					110			
Glu	Met	Ser	Leu	Glu	Glu	His	Ser	Gln	Cys	Glu	Cys	Arg	Pro	Lys	Lys	
		115					120					125				
Lys	Asp	Ser	Ala	Val	Lys	Pro	Asp	Arg	Cys	Arg	Lys	Leu	Arg	Arg		
		130					135					140				



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## (2) INFORMATION FOR SEQ ID NO:9:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 910 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 3..305

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```

CC ATG AGC CCT CTG CTC CGC CGC CTG CTG CTC GCC GCA CTC CTG CAG      47
  Met Ser Pro Leu Leu Arg Arg Leu Leu Leu Ala Ala Leu Leu Gln
    1             5             10             15

CTG GCC CCC GCC CAG GCC CCT GTC TCC CAG CCT GAT GCC CCT GGC CAC      95
  Leu Ala Pro Ala Gln Ala Pro Val Ser Gln Pro Asp Ala Pro Gly His
                20             25             30

CAG AGG AAA GTG GTG TCA TGG ATA GAT GTG TAT ACT CGC GCT ACC TGC      143
  Gln Arg Lys Val Val Ser Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys
                35             40             45

CAG CCC CGG GAG GTG GTG GTG CCC TTG ACT GTG GAG CTC ATG GGC ACC      191
  Gln Pro Arg Glu Val Val Val Pro Leu Thr Val Glu Leu Met Gly Thr
                50             55             60

GTG GCC AAA CAG CTG GTG CCC AGC TGC GTG ACT GTG CAG CGC TGT GGT      239
  Val Ala Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly
                65             70             75

GGC TGC TGC CCT GAC GAT GGC CTG GAG TGT GTG CCC ACT GGG CAG CAC      287
  Gly Cys Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr Gly Gln His
                80             85             90             95

CAA GTC CGG ATG CAG ACC TAAAAAAAAG GACAGTGCTG TGAAGCCAGA      335
  Gln Val Arg Met Gln Thr
                100

CAGGGCTGCC ACTCCCCACC ACCGTCCCCA GCCCGTTCT GTTCCGGGCT GGGACTCTGC      395

CCCCGGAGCA CCCTCCCCAG CTGACATCAC CCATCCCACT CCAGCCCCAG GCCCCTCTGC      455

CCACGCTGCA CCCAGCACCA CCAGCGCCCT GACCCCCGGA CCTGCCGCTG CCGCTGCCGA      515

CGCCGCAGCT TCCTCCGTTG CCAAGGGCGG GGCTTAGAGC TCAACCCAGA CACCTGCAGG      575

TGCCGGAAGC TGCGAAGGTG ACACATGGCT TTTCAGACTC AGCAGGGTGA CTTGCCTCAG      635

AGGCTATATC CCAGTGGGGA ACAAAGAGGA GCCTGGTAAA AAACAGCCAA GCCCCCCAAGA      695

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CCTCAGCCCA GGCAGAAGCT GCTCTAGGAC CTGGGCCTCT CAGAGGGCTC TTCTGCCATC      755
CCTTGTCTCC CTGAGGCCAT CATCAAACAG GACAGAGTTG GAAGAGGAGA CTGGGAGGCA      815
GCAAGAGGGG TCACATACCA GCTCAGGGGA GAATGGAGTA CTGTCTCAGT TTCTAACCAC      875
TCTGTGCAAG TAAGCATCTT ACAACTGGCT CTTCC                                910

```

## (2) INFORMATION FOR SEQ ID NO:10:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 101 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```

Met Ser Pro Leu Leu Arg Arg Leu Leu Leu Ala Ala Leu Leu Gln Leu
 1              5              10              15
Ala Pro Ala Gln Ala Pro Val Ser Gln Pro Asp Ala Pro Gly His Gln
          20              25              30
Arg Lys Val Val Ser Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys Gln
          35              40              45
Pro Arg Glu Val Val Val Pro Leu Thr Val Glu Leu Met Gly Thr Val
          50              55              60
Ala Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly Gly
65              70              75              80
Cys Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr Gly Gln His Gln
          85              90              95
Val Arg Met Gln Thr
          100

```

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## (2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 42 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ACCACCACCT CCCTGGGCTG GCATGTGGCA CGTGCATAAA CG

42

## (2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 42 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

AGTTGTTTGA CCACATTGCC CATGAGTTCC ATGCTCAGAG GC

42

## (2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 38 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GATCCTGGGG CTGGAGTGGG ATGGATGATG TCAGCTGG

38

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## (2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 40 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GCGGGCAGAG GATCCTGGGG CTGTCTGGCC TCACAGCACT

40

## CLAIMS:

1. A biologically isolated proteinaceous molecule having the following characteristics:
  - (i) comprises an amino acid sequence having at least about 15% similarity but at least about 5% dissimilarity to the sequence set forth in SEQ ID NO:2;
  - (ii) exhibits at least one property in common with vascular endothelial growth factor (VEGF).
2. A proteinaceous molecule according to claim 1 wherein the molecule exhibits at least one of the following properties:
  - (i) an ability to induce vascular endothelial cells;
  - (ii) an ability to interact with *flt-1/flk-1* family of receptors; and/or
  - (iii) an ability to induce cell migration, cell survival and/or an increase in intracellular levels of alkaline phosphatase.
3. A proteinaceous molecule according to claim 1 or 2 wherein said molecule has the capacity to induce astroglial proliferation.
4. A proteinaceous molecule according to claim 1 wherein said molecule is of human origin.
5. A proteinaceous molecule according to claim 1 wherein said molecule is of non-human origin.
6. A proteinaceous molecule according to claim 5 wherein said molecule is of livestock animal, companion animal, laboratory test animal, avian, fish or reptilian origin.
7. A proteinaceous molecule according to claim 5 wherein said molecule is encoded by a gene located at chromosome 11q13.

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8. A proteinaceous molecule according to claim 1 wherein the percentage similarity to SEQ ID NO:2 is at least about 30%.
9. A proteinaceous molecule according to claim 1 wherein the percentage similarity to SEQ ID NO:2 is at least about 40%.
10. A proteinaceous molecule according to claim 1 wherein the percentage similarity to SEQ ID NO:2 is at least about 60-70%.
11. A proteinaceous molecule according to claim 1 comprising a sequence of amino acids as set forth in SEQ ID NO:4 or a part, fragment, derivative or analogue thereof.
12. A proteinaceous molecule according to claim 1 comprising an amino acid sequence substantially set forth in SEQ ID NO:6 or a part, fragment, derivative or analogue thereof.
13. A proteinaceous molecule according to claim 1 comprising an amino acid sequence substantially set forth in SEQ ID NO:8 or a part, fragment, derivative or analogue thereof.
14. A proteinaceous molecule according to claim 1 comprising an amino acid sequence substantially set forth in SEQ ID NO:10 or a part, fragment, derivative or analogue thereof.
15. A recombinant molecule having the following characteristics:
  - (i) an amino acid sequence substantially as set forth in SEQ ID NO:4 or having at least about 15% similarity to but at least about 5% dissimilarity to the amino acid sequence set forth in SEQ ID NO:2;
  - (ii) exhibits at least one biological property in common with VEGF.

16. A recombinant molecule having the following characteristics:
  - (i) an amino acid sequence substantially as set forth in SEQ ID NO:6 or having at least about 15% similarity to but at least about 5% dissimilarity to the amino acid sequence set forth in SEQ ID NO:2;
  - (ii) exhibits at least one biological property in common with VEGF.
17. A recombinant molecule having the following characteristics:
  - (i) an amino acid sequence substantially as set forth in SEQ ID NO:8 or having at least about 15% similarity to but at least about 5% dissimilarity to the amino acid sequence set forth in SEQ ID NO:2;
  - (ii) exhibits at least one biological property in common with VEGF.
18. A recombinant molecule having the following characteristics:
  - (i) an amino acid sequence substantially as set forth in SEQ ID NO:10 or having at least about 15% similarity to but at least about 5% dissimilarity to the amino acid sequence set forth in SEQ ID NO:2;
  - (ii) exhibits at least one biological property in common with VEGF.
19. A recombinant molecule according to claim 15 or 16 or 17 or 18 having at least one of the following properties:
  - (a) an ability to induce vascular endothelial cells;
  - (b) an ability to interact with *flt1/flki* family of receptors;
  - (c) an ability to induce cell migration, cell survival and/or increase intracellular levels of alkaline phosphatase.
20. A recombinant molecule according to claim 15 or 16 or 17 or 18 having the capacity to induce astroglial proliferation.

21. A recombinant molecule according to claim 20 wherein the molecule comprises an amino acid sequence substantially as set forth in SEQ ID NO:6.
22. A peptide fragment corresponding to a portion of the amino acid sequence set forth in SEQ ID NO:4 or a derivative or chemical equivalent thereof.
23. A peptide fragment according to claim 22 having the sequence set forth in SEQ ID NO:6 or a chemical equivalent thereof.
24. A peptide fragment according to claim 22 having the sequence set forth in SEQ ID NO:8 or a chemical equivalent thereof.
25. A peptide fragment according to claim 22 having the sequence set forth in SEQ ID NO:10 or a chemical equivalent thereof.
26. A nucleic acid molecule comprising a sequence of nucleotides or complementary to a sequence encoding a proteinaceous molecule having the following characteristics:
- (i) comprises an amino acid sequence having at least about 15% similarity but at least about 5% dissimilarity to the sequence set forth in SEQ ID NO:2;
  - (ii) exhibits at least one property in common with vascular endothelial growth factor (VEGF).
27. A nucleic acid molecule according to claim 26 wherein the proteinaceous molecule exhibits at least one of the following properties:
- (i) an ability to induce vascular endothelial cells;
  - (ii) an ability to interact with *flt-1/flk-1* family of receptors; and/or
  - (iii) an ability to induce cell migration, cell survival and/or an increase in intracellular levels of alkaline phosphatase.
28. A nucleic acid molecule according to claim 27 wherein the proteinaceous molecule has the capacity to induce astroglial proliferation.



29. A nucleic acid molecule according to claim 28 wherein said molecule encodes an amino acid sequence substantially as set forth in SEQ ID NO:6.
30. A nucleic acid molecule according to claim 1 wherein said molecule is of human origin.
31. A nucleic acid molecule according to claim 1 wherein the percentage similarity to SEQ ID NO:2 is at least about 30%.
32. A nucleic acid molecule according to claim 26 comprising a nucleotide sequence substantially as set forth in SEQ ID NO:3 or having at least 15% similarity thereto or capable of hybridising under low stringency conditions to a reverse complement of the nucleotide sequence as set forth in SEQ ID NO:3 provided that the nucleotide sequence has at least 15% similarity but at least 30% dissimilarity to the nucleotide sequence set forth in SEQ ID NO:3.
33. A nucleic acid molecule according to claim 26 encoding a murine homologue of human VEGF and comprising a nucleotide sequence substantially as set forth in Figure 9.
34. A pharmaceutical composition comprising a proteinaceous molecule according to claim 1 or 2 or 3 or 11 and one or more pharmaceutically acceptable carriers and/or diluents.
35. A method for preparing a recombinant molecule having the following characteristics:
- (i) comprises an amino acid sequence having at least about 15% similarity but at least about 5% dissimilarity to the sequence set forth in SEQ ID NO:2;
  - (ii) exhibits at least one property in common with vascular endothelial growth factor (VEGF),
- said method comprising expressing a nucleic acid molecule encoding said recombinant

molecule by a suitable host grown under conditions effective to synthesise said recombinant molecule and then isolating said molecule.

36. A method according to claim 35 wherein the nucleic acid molecule comprises a sequence of nucleotides as set forth in SEQ ID NO:3 or having at least 15% similarity thereto or is capable of hybridising under low stringency conditions to a reverse complement of the nucleotide sequence as set forth in SEQ ID NO:3 provided that the nucleotide sequence has at least 15% similarity but at least 30% dissimilarity to the nucleotide sequence set forth in SEQ ID NO:3.

37. A method of inducing astroglial proliferation in a mammal, said method comprising administering to said mammal an effective amount of a recombinant proteinaceous molecule having the characteristics:

- (i) comprises an amino acid sequence having at least about 15% similarity but at least about 5% dissimilarity to the sequence set forth in SEQ ID NO:2;
- (ii) exhibits at least one property in common with vascular endothelial growth factor (VEGF),

said administration being for a time and under conditions sufficient to induce astroglial proliferation.

38. A method according to claim 37 wherein the recombinant proteinaceous molecule comprises an amino acid sequence substantially as set forth in SEQ ID NO:3 or is a derivative thereof.

39. A method according to claim 37 wherein the recombinant proteinaceous molecule comprises an amino acid sequence substantially as set forth in SEQ ID NO:6 or is a derivative thereof.

40. A method of promoting neuronal survival and/or proliferation in a mammal, said method comprising administering to said mammal an effective amount of a recombinant proteinaceous molecule having the characteristics:

- 57 -

- (i) comprises an amino acid sequence having at least about 15% similarity but at least about 5% dissimilarity to the sequence set forth in SEQ ID NO:2;
- (ii) exhibits at least one property in common with vascular endothelial growth factor (VEGF),

said administration being for a time and under conditions sufficient to induce astroglial proliferation.

41. A method according to claim 40 wherein the recombinant proteinaceous molecule comprises an amino acid sequence substantially as set forth in SEQ ID NO:3 or is a derivative thereof.

41. A method according to claim 40 wherein the recombinant proteinaceous molecule comprises an amino acid sequence substantially as set forth in SEQ ID NO:6 or is a derivative thereof.

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<i>2/52</i>  <i>Fig.1(i)</i>	<i>3/52</i>  <i>Fig.1(ii)</i>
<i>4/52</i>  <i>Fig.1(iii)</i>	<i>5/52</i>  <i>Fig.1(iv)</i>

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1	TCGGCCTCC	GAAACC	ATG	AAC	TTT	CTG		
			Met	Asn	Phe	Leu		
			1					
50	CTT	GCC	TTG	CTG	CTC	TAC	CTC	CAC
	Leu	Ala	Leu	Leu	Leu	Tyr	Leu	His
			15					
98	CCC	ATG	GCA	GAA	GGA	GGA	GGG	CAG
	Pro	Met	Ala	Glu	Gly	Gly	Gly	Gln
			30					35
146	ATG	GAT	GTC	TAT	CAG	CGC	AGC	TAC
	Met	Asp	Val	Tyr	Gln	Arg	Ser	Tyr
		45					50	
194	GAC	ATC	TTC	CAG	GAG	TAC	CCT	GAT
	Asp	Ile	Phe	Gln	Glu	Tyr	Pro	Asp
	60					65		
242	TCC	TGT	GTG	CCC	CTG	ATG	CGA	TGC
	Ser	Cys	Val	Pro	Leu	Met	Arg	Cys
					80			
290	CTC	GAG	TGT	GTG	CCC	ACT	GAG	GAG
	Leu	Glu	Cys	Val	Pro	Thr	Glu	Glu
					95			
338	CGG	ATC	AAA	CCT	CAC	CAA	GGC	CAG
	Arg	Ily	Lys	Pro	His	Gln	Gly	Gln
			110					115

Fig.1(i)

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CTG	TCT	TGG	GTG	CAT	TGG	AGC		49
Leu	Ser	Trp	Val	His	Trp	Ser		
5					10			
CAT	GCC	AAG	TGG	TCC	CAG	GCT	GCA	97
His	Ala	Lys	Trp	Ser	Gln	Ala	Ala	
20					25			
AAT	CAT	CAC	GAA	GTG	GTG	AAG	TTC	145
Asn	His	His	Glu	Val	Val	Lys	Phe	
			40					
TGC	CAT	CCA	ATC	GAG	ACC	CTG	GTG	193
Cys	His	Pro	Ile	Glu	Thr	Leu	Val	
			55					
GAG	ATC	GAG	TAC	ATC	TTC	AAG	CCA	241
Glu	Ile	Glu	Tyr	Ile	Phe	Lys	Pro	
		70					75	
GGG	GGC	TGC	TGC	AAT	GAC	GAG	GGC	289
Gly	Gly	Cys	Cys	Asn	Asp	Glu	Gly	
	85					90		
TCC	AAC	ATC	ACC	ATG	CAG	ATT	ATG	337
Ser	Asn	Ile	Thr	Met	Gln	Ile	Met	
100					105			
CAC	ATA	GGA	GAG	ATG	AGC	TTC	CTA	385
His	Ile	Gly	Glu	Met	Ser	Phe	Leu	
			120					

Fig.1(ii)

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386	CAG CAC AAC AAA TGT GAA TGC AGA
	Gln His Asn Lys Cys Glu Cys Arg
	125 130
434	GAA AAT CCC TGT GGG CCT TGC TCA
	Glu Asn Pro Cys Gly Pro Cys Ser
	140 145
482	CAA GAT CCG CAG ACG TGT AAA TGT
	Gln Asp Pro Gln Thr Cys Lys Cys
	160
530	TGC AAG GCG AGG CAG CTT GAG TTA
	Cys Lys Ala Arg Gln Leu Glu Leu
	175
578	AAG CCG AGG CGG TGAGCCGGGC AGGAG
	Lys Pro Arg Arg
	190
630	GAACCAGATC TCTCACCAGG

*Fig.1(iii)*

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CCA	AAG	AAA	GAT	AGA	GCA	AGA	CAA	433
Pro	Lys	Lys	Asp	Arg	Ala	Arg	Gln	
			135					
GAG	CGG	AGA	AAG	CAT	TTG	TTT	GTA	481
Glu	Arg	Arg	Lys	His	Leu	Phe	Val	
		150					155	
TCC	TGC	AAA	AAC	ACA	GAC	TCG	CGT	529
Ser	Cys	Lys	Asn	Thr	Asp	Ser	Arg	
	165					170		
AAC	GAA	CGT	ACT	TGC	AGA	TGT	GAC	577
Asn	Glu	Arg	Thr	Cys	Arg	Cys	Asp	
180					185			
GAAGG	AGCCTCCCTC	AGCGTTTCGG						629
								649

*Fig.1(iv)*



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<i>7/52</i>  <i>Fig.2(i)</i>	<i>8/52</i>  <i>Fig.2(ii)</i>
<i>9/52</i>  <i>Fig 2(iii)</i>	<i>10/52</i>  <i>Fig 2(iv)</i>
<i>11/52</i>  <i>Fig 2(v)</i>	<i>12/52</i>  <i>Fig 2(vi)</i>

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1	CC	ATG	AGC	CCT	CTG	CTC	CGC	CGC
	Met	Ser	Pro	Leu	Leu	Arg	Arg	
	1					5		
48	CTG	GCC	CCC	GCC	CAG	GCC	CCT	GTC
	Leu	Ala	Pro	Ala	Gln	Ala	Pro	Val
					20			
96	CAG	AGG	AAA	GTG	GTG	TCA	TGG	ATA
	Gln	Arg	Lys	Val	Val	Ser	Trp	Ile
				35				
144	CAG	CCC	CGG	GAG	GTG	GTG	GTG	CCC
	Gln	Pro	Arg	Glu	Val	Val	Val	Pro
			50					55
192	GTG	GCC	AAA	CAG	CTG	GTG	CCC	AGC
	Val	Ala	Lys	Gln	Leu	Val	Pro	Ser
		65					70	
240	GGC	TGC	TGC	CCT	GAC	GAT	GGC	CTG
	Gly	Cys	Cys	Pro	Asp	Asp	Gly	Leu
	80					85		
288	CAA	GTC	CGG	ATG	CAG	ATC	CTC	ATG
	Gln	Val	Arg	Met	Gln	Ile	Leu	Met
				100				
336	GGG	GAG	ATG	TCC	CTG	GAA	GAA	CAC
	Gly	Glu	Met	Ser	Leu	Glu	Glu	His
				115				

Fig.2(i)

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CTG	CTG	CTC	GCC	GCA	CTC	CTG	CAG	47
Leu	Leu	Leu	Ala	Ala	Leu	Leu	Gln	
		10					15	
TCC	CAG	CCT	GAT	GCC	CCT	GGC	CAC	95
Ser	Gln	Pro	Asp	Ala	Pro	Gly	His	
	25					30		
GAT	GTG	TAT	ACT	CGC	GCT	ACC	TGC	143
Asp	Val	Tyr	Thr	Arg	Ala	Thr	Cys	
40					45			
TTG	ACT	GTG	GAG	CTC	ATG	GGC	ACC	191
Leu	Thr	Val	Glu	Leu	Met	Gly	Thr	
			60					
TGC	GTG	ACT	GTG	CAG	CGC	TGT	GGT	239
Cys	Val	Thr	Val	Gln	Arg	Cys	Gly	
			75					
GAG	TGT	GTG	CCC	ACT	GGG	CAG	CAC	287
Glu	Cys	Val	Pro	Thr	Gly	Gln	His	
		90					95	
ATC	CGG	TAC	CCG	AGC	AGT	CAG	CTG	335
Ile	Arg	Tyr	Pro	Ser	Ser	Gln	Leu	
	105					110		
AGC	CAG	TGT	GAA	TGC	AGA	CCT	AAA	383
Ser	Gln	Cys	Glu	Cys	Arg	Pro	Lys	
120					125			

Fig. 2(ii)

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384	AAA	AAG	GAC	AGT	GCT	GTG	AAG	CCA
	Lys	Lys	Asp	Ser	Ala	Val	Lys	Pro
			130					135
432	CGT	CCC	CAG	CCC	CGT	TCT	GTT	CCG
	Arg	Pro	Gln	Pro	Arg	Ser	Val	Pro
		145					150	
480	CCC	TCC	CCA	GCT	GAC	ATC	ACC	CAT
	Pro	Ser	Pro	Ala	Asp	Ile	Thr	His
	160					165		
528	GCC	CAC	GCT	GCA	CCC	AGC	ACC	ACC
	Ala	His	Ala	Ala	Pro	Ser	Thr	Thr
				180				
576	GCT	GCC	GCT	GCC	GAC	GCC	GCA	GCT
	Ala	Ala	Ala	Ala	Asp	Ala	Ala	Ala
			195					

*Fig. 2(iii)*

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GAC	AGG	GCT	GCC	ACT	CCC	CAC	CAC	431
Asp	Arg	Ala	Ala	Thr	Pro	His	His	
				140				
GGC	TGG	GAC	TCT	GCC	CCC	GGA	GCA	479
Gly	Trp	Asp	Ser	Ala	Pro	Gly	Ala	
				155				
CCC	ACT	CCA	GCC	CCA	GGC	CCC	TCT	527
Pro	Thr	Pro	Ala	Pro	Gly	Pro	Ser	
				170			175	
AGC	GCC	CTG	ACC	CCC	GGA	CCT	GCC	575
Ser	Ala	Leu	Thr	Pro	Gly	Pro	Ala	
				185			190	
TCC	TCC	GTT	GCC	AAG	GGC	GGG	GCT	T 624
Ser	Ser	Val	Ala	Lys	Gly	Gly	Ala	
200					205			

Fig. 2(iv)

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625	AGAGCTCAAC	CCAGACACCT	GCAGGTGCCG
685	GACTCAGCAG	GGTGACTTGC	CTCAGAGGCT
745	GGTAAAAAAC	AGCCAAGCCC	CCAAGACCTC
805	GCCTCTCAGA	GGGCTCTTCT	GCCATCCCTT
865	GAGTTGGAAG	AGGAGACTGG	GAGGCAGCAA
825	GGAGTACTGT	CTCAGTTTCT	AACCACTCTG
985	CTCCCCTCAC	TAAGAAGACC	CAAACCTCTG
1045	CTGTGACCCC	CAACCCTGAT	AAAAGAGATG

*Fig.2(v)*

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GAAGCTGCGA	AGGTGACACA	TGGCTTTTCA	684
ATATCCCAGT	GGGGGAACAA	AGGGGAGCCT	744
AGCCCAGGCA	GAAGCTGCTC	TAGGACCTGG	804
GTCTCCCTGA	GGCCATCATC	AAACAGGACA	864
GAGGGGTCAC	ATACCAGCTC	AGGGGAGAAT	924
TGCAAGTAAG	CATCTTACAA	CTGGCTCTTC	984
CATAATGGGA	TTTGGGCTTT	GGTACAAGAA	1044
GAAGGAAAAA	AAAAAAAAAA		1094

*Fig.2(vi)*

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<p>14/52</p> <p><i>Fig. 3(i)</i></p>	<p>15/52</p> <p><i>Fig. 3(ii)</i></p>
--------------------------------------	---------------------------------------



14/52

>VEGF\_HUMAN VEGF\_HUMAN VASCULAR ENDOTHELIAL  
(VASCULAR 215 AA.  
LENGTH = 215

SCORE = 181 (92.4 BITS), EXPECT = 6.4e-20,  
IDENTITIES = 33/75 (44%), POSITIVES = 48/75

QUERY: 31 HQRKVVSVIDVYTRATCQPREVVVPLTVEL  
+++ VV +DVY R+ C+P E +V + E  
SBJCT: 36 NHHEVVKFMDVYQRSYCHPIETLVDIFQEY

QUERY: 91 PTGQHQVRMQILMIR 105  
PT + + MQI+ I+  
SBJCT: 96 PTEESNITMQIMRIK 110

SCORE = 76 (38.8 BITS), EXPECT = 0.0011,  
IDENTITIES = 12/19 (63%), POSITIVES = 16/19

QUERY: 110 QLGEMSLEEHSQCECRPKK 128  
++GEMS +H+ CECRPKK  
SBJCT: 116 HIGEMSFLQHNKCECRPKK 134

SCORE = 72 (36.8 BITS), EXPECT = 0.0046,  
IDENTITIES = 14/21 (66%), POSITIVES = 15/21

QUERY: 202 RCQGRGLELNPDTCTCRCLRR 222  
RC +R LELN TCRC K RR  
SBJCT: 195 RCKARQLELNERTCRCDKPRR 215

SCORE = 46 (23.5 BITS), EXPECT = 47.,  
IDENTITIES = 6/10 (60%), POSITIVES = 9/10

QUERY: 187 DPRTCTCRCTCR 196  
DP+TC+C C+  
SBJCT: 181 DPQTCKCSCK 190

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*Fig.3(i)*

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GROWTH FACTOR PRECURSOR (VEGF)

 $P = 6.4e-20$   
(64%)

MGTVAKQLVPSCVTVQRCGGCCPDDGLECV 90  
+ PSCV + RCGGCC D+GLECV  
PDEIEYIFKPSCVPLMRCGGCCNDEGLECV 95

 $\text{POISSON } P(2) = 9.1e-12$   
(84%) $\text{POISSON } P(3) = 3.6e-18$   
(71%) $\text{POISSON } P(4) = 7.3e-10$   
(90%)*Fig. 3(i)*

*16/52*

<i>17/52</i>  <i>Fig.4(i)</i>	<i>18/52</i>  <i>Fig.4(ii)</i>
<i>19/52</i>  <i>Fig.4(iii)</i>	<i>20/52</i>  <i>Fig.4(iv)</i>

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Gap Weight:3.00	Average Match:1.000
Length Weight:0.100	Average Mismatch:-0.900
Quality:100.9	Length:739
Ratio:0.175	Gaps:30
Percent	Percent
Similarity:69.703	Identity:69.703

```

28      ATGAGCCCTCTGCTCCGCCGCCTGC
        |||| | ||||| | ||
17      ATGAACTTTCTGCT.....GTCT..

68      TGCAGCTGGCCCCCGCCCAGGCCCC
        ||| ||| || | ||| |||
57      TGCTGCTCTACCTCCACCATGCCAA

118     CACCAGAGGA.....
          |||||
106     AGAAGGAGGAGGGCAGAATCATCAC

140     GTGTATACTCGC.GCTACCTGCCAG
        || ||| ||| ||| |||||
152     GTCTATCAGCGCAGCTA.CTGCCAT

194     T....GA.....CTGTGGAGCTCAT
        | || ||| ||| ||
201     TCCAGGAGTACCCTGATGAGATCGA

235     CCCAGCTGCGTGACTGTGCAGCGCT
        || ||| ||| | ||| |
239     CCATCCTGTGTGCCCCCTGATGCGAT

285     CCTGGAGTGTGTGCCCCACTGGGCAG
        ||||| ||||| ||||| |||
289     CCTGGAGTGTGTGCCCCACTGAGGAG

```

Fig.4(i)

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TGCTCGCCGCACT.....CC	67
...TGGGTGCATTGGAGCCTTGCCT	56
TGTCTCCCAGCCTGATGCCCCTGGC	117
GTGGTCCCAGGCTGCA.CCCATGGC	105
.AAGTGGTG....TCATGGATAGAT	147
GAAGTGGTGAAGTTCATG....GAT	151
CCCCGGGAG...GTGGTGGTGCCCT	193
CCAATCGAGACCCTGGTGGACATCT	200
GGGCACCGTGGCCAAACAGCTGGTG	234
GTACATCTT...CAA.....G	238
GTGGTGGCTGCTGCCCTGACGATGG	284
GCGGGGGCTGCTGCAATGACGAGGG	288
CACCAAGTCCGGATGCAGAT.....	329
TCCAACATCACCATGCAGATTATGC	338

*Fig.4(ii)*  
SUBSTITUTE SHEET (RULE 26)

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330 . . . . . CCTCATGATCCGGTACC  
                    |||||  
339 GGATCAAACCTCA . . . . . C  
  
369 GTCCCTGGAAGAACACAGCCAGTGT  
     |   |   |     |   |   |||   |   |   |||  
376 GAGCTTCCTACAGCACAAACAATGT  
  
419 GTGCTGTGAAGCCAGACAGGGCTGC  
     |                |||   |||   |   |  
423 G . . . . . AGCAAGACAAG . . . . .  
  
469 CGTTCTGTTCCGGGCTGGGACTCTG  
         |   |   ||   |||   ||  
443 . . . TGTGGGCCTTGCTCAGA . . . . .  
  
519 CATCACCCATCCC ACTCCAGCCCCA  
  
468 . . . . .  
  
569 GC . . . . . ACCACCAGCGCCC  
     ||                  ||   |  
469 GCATTTGTTTGTACAA . . . . .  
  
609 TGCCGACGCCGCAGCTTCCTCCGTT  
     ||   |   |     |   |||   ||   |||  
509 TG . CAAAAACACAGACTC . . GCGTT  
  
657 AACCCAGACACCTGCAGGTGCCGGA  
     |||   |  
554 AACGAACGTACTTGCAGATGTGACA

Fig. 4 (iii)

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CGAGCAGTCAGC . . . TGGGGGAGAT	368
CAAG . . GCCAGCACATAGGAGAGAT	375
GAATGCAGACCTAAAAAAAAAGGACA	418
GAATGCAGACC . . . AAAGAAAGATA	422
CACTCCCCACCACCGTCCCCAGCCC	468
. . . . . AAAATCCC . . . . .	442
CCCCCGGAGCACCTCCCCAGCTGA	518
. . . GCGGAGAA . . . . .	467
GGCCCCTCTGCCCACGCTGCACCCA	568
. . . . . A	468
TGACCCCCGGACCTGCCGCTGCCGC	608
. GATCCGCAGACGTGTAAATGTTCC	508
GCCAAGGGCGGGGC . . TTAGAGCTC	656
GC . . AAGGCGAGGCAGCTTGAGTTA	553
AGCTGCGAAGGTGA	695
AGCCGAGGCGGTGA	592

Fig.4(iv)

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<i>22/52</i>  <i>Fig.5(i)</i>	<i>23/52</i>  <i>Fig.5(ii)</i>	<i>24/52</i>  <i>Fig.5(iii)</i>
<i>25/52</i>  <i>Fig.5(iv)</i>	<i>26/52</i>  <i>Fig.5(v)</i>	<i>27/52</i>  <i>Fig.5(vi)</i>



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165SOMSQ.MSF.msf MSF:687

Type: D Tuesday, June 20, 1995

Check:3140

	1
VEGF165	ATGAACTTTCTGCTGTCTTGGGTG
SOM175	ATGAGCCCTCTGCTCCGCCGCCTG
SOM175-e6	ATGAGCCCTCTGCTCCGCCGCCTG
SOM175-e6&7	ATGAGCCCTCTGCTCCGCCGCCTG
SOM175-e4	ATGAGCCCTCTGCTCCGCCGCCTG
	81
VEGF165	CACCCATGGCAGAAGGAGGAGGGC
SOM175	TGCCCCCTGGCCACCAGAGGAAAGT
SOM175-e6	TGCCCCCTGGCCACCAGAGGAAAGT
SOM175-e6&7	TGCCCCCTGGCCACCAGAGGAAAGT
SOM175-e4	TGCCCCCTGGCCACCAGAGGAAAGT
	161
VEGF165	CCAATCGAGACCCTGGTGGACATC
SOM175	GTGGTGGTGCCCTTGACTG.TGGA
SOM175-e6	GTGGTGGTGCCCTTGACTG.TGGA
SOM175-e6&7	GTGGTGGTGCCCTTGACTG.TGGA
SOM175-e4	GTGGTGGTGCCCTTGACTG.TGGA
	241
VEGF165	GATGCGATGCGGGGGGCTGCTGCAA
SOM175	GCAGCGCTGTGGTGGCTGCTGCCC
SOM175-e6	GCAGCGCTGTGGTGGCTGCTGCCC
SOM175-e6&7	GCAGCGCTGTGGTGGCTGCTGCCC
SOM175-e4	GCAGCGCTGTGGTGGCTGCTGCCC

*Fig.5(i)*

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CATTGGAGCCTTGCCTTGCTGCTCTACC  
CTGCTCGCCGCACTCCTGCAGCTGGCCC  
CTGCTCGCCGCACTCCTGCAGCTGGCCC  
CTGCTCGCCGCACTCCTGCAGCTGGCCC  
CTGCTCGCCGCACTCCTGCAGCTGGCCC

AGAATCATCACGAAGTGGTGAAGTTCAT  
GGTGTCATGGATAGATGTGTATACTCGC  
GGTGTCATGGATAGATGTGTATACTCGC  
GGTGTCATGGATAGATGTGTATACTCGC  
GGTGTCATGGATAGATGTGTATACTCGC

TTCCAGGAGTACCCTGATGAGATCGAGT  
GCTCATGGGCACCGTGGCCAAAC..AGC  
GCTCATGGGCACCGTGGCCAAAC..AGC  
GCTCATGGGCACCGTGGCCAAAC..AGC  
GCTCATGGGCACCGTGGCCAAAC..AGC

TGACGAGGGCCTGGAGTGTGTGCCCCACT  
TGACGATGGCCTGGAGTGTGTGCCCCACT  
TGACGATGGCCTGGAGTGTGTGCCCCACT  
TGACGATGGCCTGGAGTGTGTGCCCCACT  
TGACGATGGCCTGGAGTGTGTGCCCCACT

*Fig. 5(ii)*

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80

TCCACCATGCCAAGTGGTCCCAGGCTG.  
CCGCCCAGGCCCCTGTCTCCCAGCCTGA  
CCGCCCAGGCCCCTGTCTCCCAGCCTGA  
CCGCCCAGGCCCCTGTCTCCCAGCCTGA  
CCGCCCAGGCCCCTGTCTCCCAGCCTGA

160

GGATGTCTATCAGCGCAGCTACTGCCAT  
G.....CTACCTGC.CAGCC.CCGGGAG  
G.....CTACCTGC.CAGCC.CCGGGAG  
G.....CTACCTGC.CAGCC.CCGGGAG  
G.....CTACCTGC.CAGCC.CCGGGAG

240

ACATCTTCAAGCCATCCTGTGTGCCCCCT  
TGGTGCCCAG.....CTGCGTGA CTGT  
TGGTGCCCAG.....CTGCGTGA CTGT  
TGGTGCCCAG.....CTGCGTGA CTGT  
TGGTGCCCAG.....CTGCGTGA CTGT

320

GAGGAGTCCAACATCACCATGCAGATTA  
GGGCAGCACCAAGTCCGGATGCAGATCC  
GGGCAGCACCAAGTCCGGATGCAGATCC  
GGGCAGCACCAAGTCCGGATGCAGATCC  
GGGCAGCACCAAGTCCGGATGCAGA...

*Fig.5(iii)*

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	321
VEGF165	TGCGGATCAAACCTCACCAAGGCC
SOM175	TCATGATCCGG...TACCCGAGCA
SOM175-e6	TCATGATCCGG...TACCCGAGCA
SOM175-e6&7	TCATGATCCGG...TACCCGAGCA
SOM175-e4	.....
	401
VEGF165	AAGAAAGATAG.....AGCAA
SOM175	AAAAAGGACAGTGCTGTGAAGCCA
SOM175-e6	AAAAAGGACAGTGCTGTGAAGCCA
SOM175-e6&7	AAAAAGGACAGTGCTGTGAAGCCA
SOM175-e4	AAAAAGGACAGTGCTGTGAAGCCA
	481
VEGF165	.....AAGCA.....
SOM175	CTCTGCCCCCGGAGCACCCCTCCCC
SOM175-e6	.....
SOM175-e6&7	.....
SOM175-e4	CTCTGCCCCCGGAGCACCCCTCCCC
	561
VEGF165	A.....GATCCGCA
SOM175	GCACCACCAGCGCCCTGACCCCCG
SOM175-E6	GCACCACCAGCGCCCTGACCCCCG
SOM175-e6&7	.....
SOM175-e4	GCACCACCAGCGCCCTGACCCCCG
	641
VEGF165	TTGAGTTAAACGAACGTACTTGCA
SOM175	TAGAGCTCAACCCAGACACCTGCA
SOM175-e6	TAGAGCTCAACCCAGACACCTGCA
SOM175-e6&7	.....
SOM175-e4	TAGAGCTCAACCCAGACACCTGCA

Fig.5(iv)

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AGCACATAGGAGAGATGAGCTTCCTACA  
GTCAGCTGGGGGAGATGTCCCTGGAAGA  
GTCAGCTGGGGGAGATGTCCCTGGAAGA  
GTCAGCTGGGGGAGATGTCCCTGGAAGA  
.....

GACAAGAA....AATCCCTGTGG.....  
GACAGGGCTGCCACTCCCCACCACCGTC  
GATAG.....  
GATAG.....  
GACAGGGCTGCCACTCCCCACCACCGTC

.....  
AGCTGACATCACCCATCCCCTCCAGCC  
.....CC  
.....  
AGCTGACATCACCCATCCCCTCCAGCC

GACGTGTAAATGTTCTCTGCAAAAAC.AC  
GACCTGCCGCTGCCGCTGCCGACGCCGC  
GACCTGCCGCTGCCGCTGCCGACGCCGC  
.....  
GACCTGCCGCTGCCGCTGCCGACGCCGC

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GATGTGACAAGCCGAGGCGGTGA  
GGTGCCGGAAGCTGCGAAGGTGA  
GGTGCCGGAAGCTGCGAAGGTGA  
.GTGCCGGAAGCTGCGAAGGTGA  
GGTGCCGGAAGCTGCGAAGGTGA

*Fig.5(v)*

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400  
GCACAACAAATGTGAATGCAGACC...A  
ACACAGCCAGTGTGAATGCAGACCTAAA  
ACACAGCCAGTGTGAATGCAGACCTAAA  
ACACAGCCAGTGTGAATGCAGACCTAAA  
.....CCTAAA

480  
.....GCCTTGCTCAGAGCGGAGA  
CCCAGCCCCGTTCTGTTCCGGGCTGGGA  
.....  
.....  
CCCAGCCCCGTTCTGTTCCGGGCTGGGA

560  
.....TTTGTT.....TGTAC..A  
CCAGGCCCCCTCTGCCCACGCTGCACCCA  
CCAGGCCCCCTCTGCCCACGCTGCACCCA  
.....  
CCAGGCCCCCTCTGCCCACGCTGCACCCA

640  
AGACTCG..CGTTGCAAGGCGAGGCAGC  
AGCTTCCTCCGTTGCCAAGGGCGGGGCT  
AGCTTCCTCCGTTGCCAAGGGCGGGGCT  
.....  
AGCTTCCTCCGTTGCCAAGGGCGGGGCT

*Fig.5(vi)*

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<i>Fig 6(i)</i>	29/52	<i>Fig 6(ii)</i>	30/52
<i>Fig 6(iii)</i>	31/52		

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VEGF <sub>165</sub>	M	N	F	L	L	S	W	V	H	W	S	L	A	L	L	L	Y	L	H	H	A	K	W	S	Q	A	A	P
SOM175 <sub>Short</sub>	M	S	P	L	L	R	R	L	.	.	.	L	A	A	L	L	Q	L	A	P	A	Q	.	.	.	.	A	P
VEGF <sub>165</sub>	I	F	Q	E	Y	P	D	E	I	E	Y	I	F	K	P	S	C	V	P	L	M	R	C	G	G	C	C	N
SOM175 <sub>Short</sub>	L	T	V	E	L	M	G	T	V	A	K	Q	L	V	P	S	C	V	T	V	Q	R	C	G	G	C	C	P
VEGF <sub>165</sub>	F	L	Q	H	N	K	C	E	C	R	P	K	K	.	.	.	.	.	.	.	D	R	A	.	.	.	.	.
SOM175 <sub>Short</sub>	L	E	E	H	S	Q	C	E	C	R	P	K	K	K	D	S	A	V	K	P	D	R	A	A	T	P	H	
VEGF <sub>165</sub>	C	K	C	S	C	K	N	T	D	S	R	C	K	A	R	Q	L	E	L	N	E	R	T	C	R	C	D	K
SOM175 <sub>Short</sub>	H	A	A	P	S	T	S	A	L	T	P	G	P	A	A	A	A	A	A	D	A	A	S	S	V	A	K	
OR...																												
VEGF <sub>165</sub>	M	N	F	L	L	S	W	V	H	W	S	L	A	L	L	Y	L	H	H	A	K	W	S	Q	A	A	P	
SOM175 <sub>Long</sub>	M	S	P	L	L	R	R	L	.	.	.	L	A	A	L	L	Q	L	A	P	A	Q	.	.	.	.	A	P
VEGF <sub>165</sub>	I	F	Q	E	Y	P	D	E	I	E	Y	I	F	K	P	S	C	V	P	L	M	R	C	G	G	C	C	N
SOM175 <sub>Long</sub>	L	T	V	E	L	M	G	T	V	A	K	Q	L	V	P	S	C	V	T	V	Q	R	C	G	G	C	C	P
VEGF <sub>165</sub>	F	L	Q	H	N	K	C	E	C	R	P	K	K	.	.	.	.	.	.	.	D	R	A	.	.	.	.	.
SOM175 <sub>Long</sub>	L	E	E	H	S	Q	C	E	C	R	P	K	K	K	D	S	A	V	K	P	D	R	A	A	T	P	H	
VEGF <sub>165</sub>	G	P	C	S	E	R	R	K	H	L	F	V	Q	D	P	Q	T	C	K	C	S	C	K	N	T	D	S	.
SOM175 <sub>Long</sub>	P	R	C	T	Q	H	Q	R	.	.	.	P	D	P	R	T	C	R	C	R	C	R	R	R	S	F	L	.

Fig.6(ii)



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MAEGGQN HHE . VVKFM DVYQ RSYC HPC IETLV D 60  
 VSQPDAPGH QRK VV VSWI DVYT RARCTC PR E VVP 55

DEGLCEVP T EESNI TMQIMRI KPHQGQH I GEMS 121  
 DDEGLCEVP T GQH QV RMQ ILMIR . YPSSQL GEMS 115

. . . . . RQEN PCGP CSERR KH LF . VQDP Q T 170  
 RPQPRSVPGWDSA PP GAPP SPADIT HP TPA GP SA 175

PRR  
 GGA

191  
 207

MAEGGQN HHE . VVKFM DVYQ RSYC HPC IETLV D 60  
 VSQPDAPGH QRK VV VSWI DVYT RARCTC PR E VVP 55

DEGLCEVP T EESNI TMQIMRI KPHQGQH I GEMS 121  
 DDEGLCEVP T GQH QV RMQ ILMIR . YPSSQL GEMS 115

RQENP . . . . . SAPGAPSPADIT HPTPA GP L C 170  
 RPQPRSVPGWDSA . . . . . C 177

RCKARQL E L N ER TCRC DKP RR 191  
 RCQGRGL E L N PD TCRC KLR R 222

Fig. 6(ii)

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Areas of 100% homology are boxed and conserved residues thought to be involved in homodimerisation are underlined. The VEGF sequence depicted includes the 26 amino acid leader sequence (removal of which gives rise to mature VEGF<sub>165</sub>) giving a total length of 191 amino acids.

Homology of SOM175 to VEGF<sub>165</sub> is 27% (33%) at the protein level, however within this are blocks of 100% homology. In particular, many structural residues are conserved including those thought to be involved in homodimerisation of VEGF (by comparison with PDGF).  
ie. Cysteine-47

Proline-70, Cysteine-72, Valine-74  
Arginine-77, Cystein-78, Glycine-80, Cysteines-81 & 82  
Cysteine-89, Proline-91  
Cysteines 122 & 124

*Fig.6(iii)*

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SPLICE VARIANTS OF SOM175

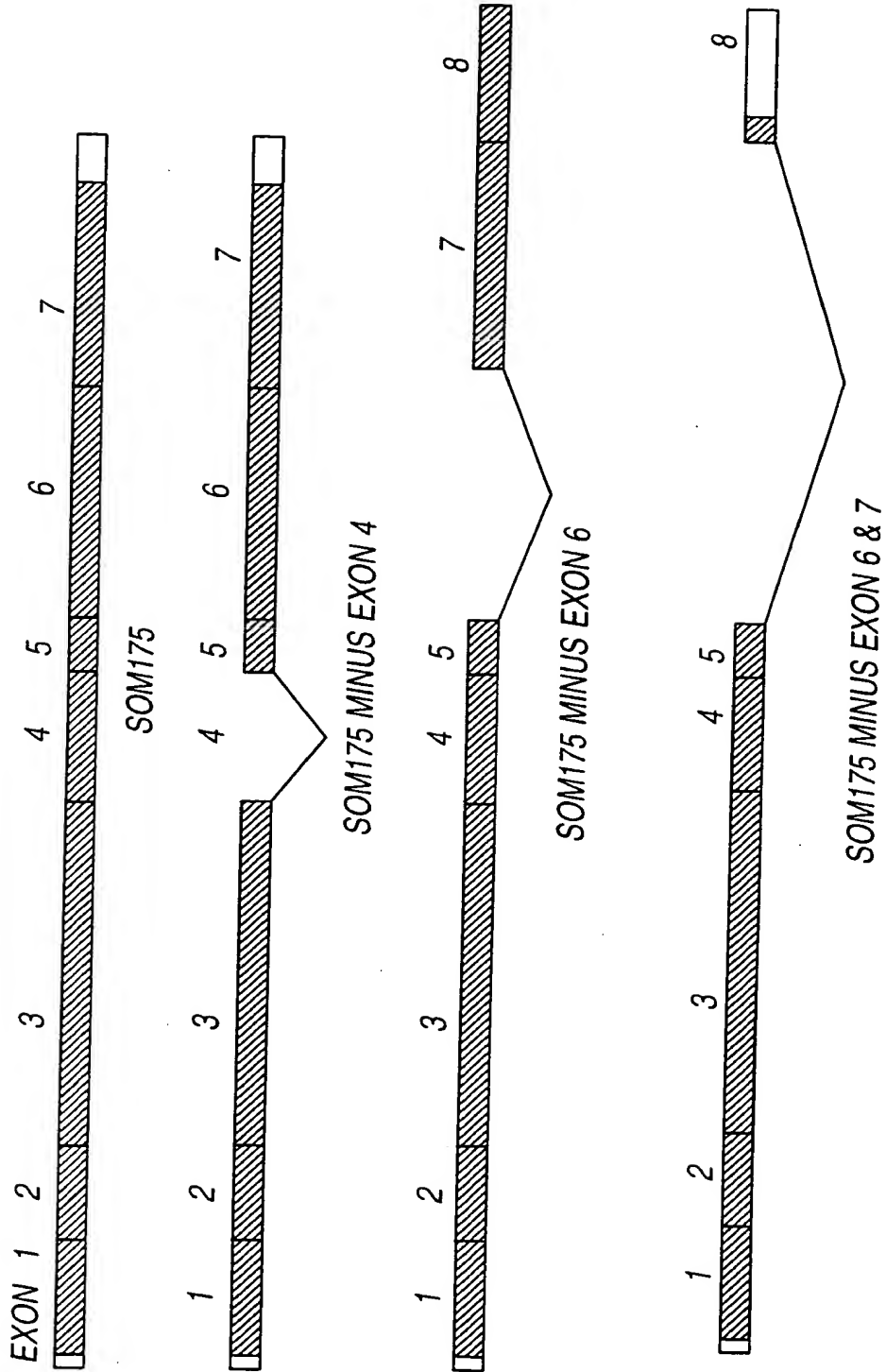


Fig. 7

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## GENOMIC STRUCTURE OF HUMAN SOM175

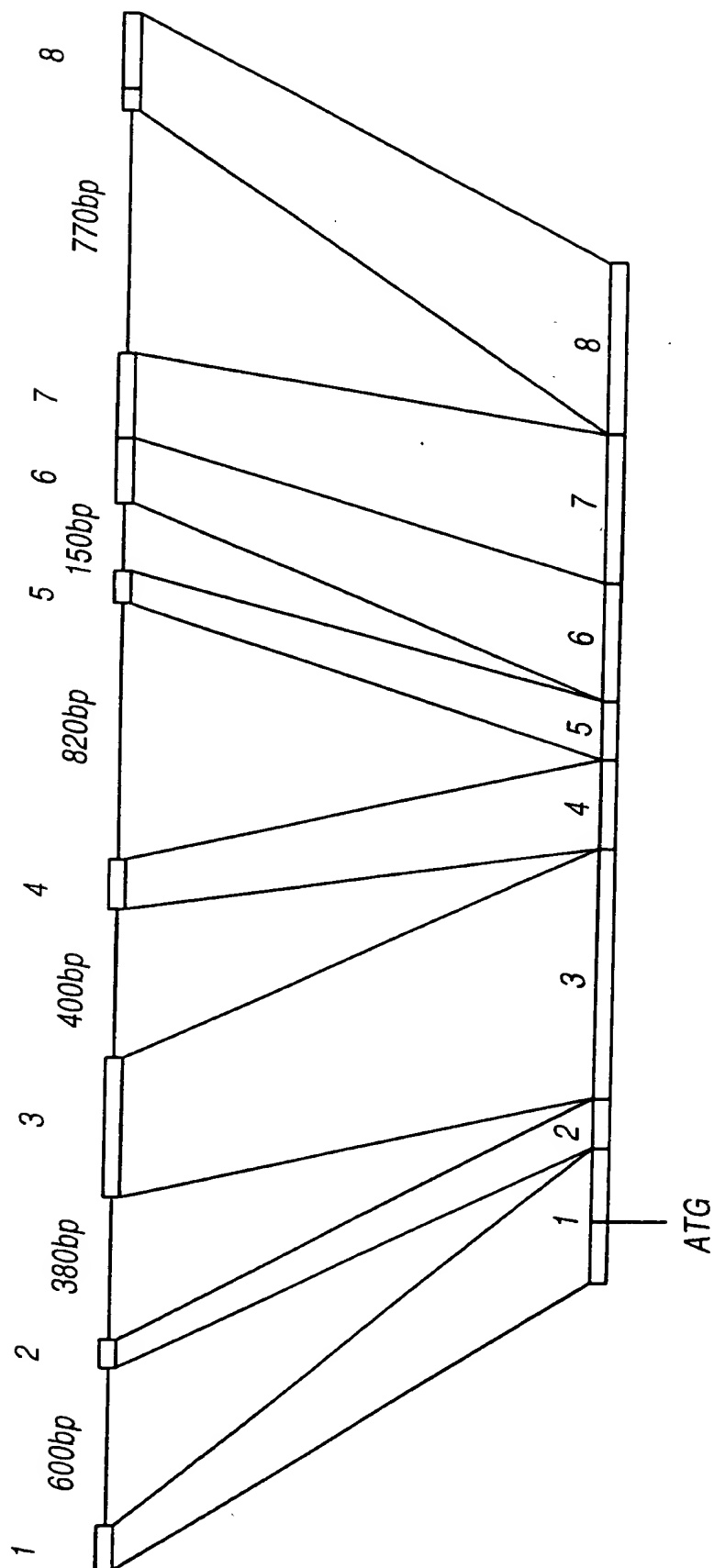


Fig. 8A

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5'UTR... <b>ATGAGG</b>	*Exon 1 (60bp)	GGCCAG gtacgtgagg
tctccacag GCCCCT	Exon 2 (43bp)	GGAAG aatactaca
tctgctcca TGGTGT	Exon 3 (187bp)	ATGCAG gtccgagatg
ctgaatacag ATCCTC	Exon 4 (73bp)	ATGCAG gtgtcaggca
acttttcaag ACCTAA	Exon 5 (34bp)	AGACAG gtgagtcttt
ctcctccgta GGCTGC	Exon 6 (101bp)	CTCCAG cccaggccc
cccactccag CCCCAG	Exon 7 (109bp)	ACCCAG acacctgtag
ccctgctcag GTGCCG	*Exon 8 (22bp)	AGG <b>TGA</b> ...3'UTR

*Fig.8B*

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<i>36/52</i>  <i>Fig. 9(i)</i>	<i>37/52</i>  <i>Fig. 9(ii)</i>
<i>38/52</i>  <i>Fig. 9(iii)</i>	<i>39/52</i>  <i>Fig. 9(iv)</i>

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-163	gcacgagctcaggccgctcgctgcggcgctg
-103	gggggcccgcggaggagccgccccctgcgcc
-43	ggcggctctggctgacccccccccacaccg
16	CGTCGCCTGCTGCTTGTTGCACTGCTGCAG
	R R L L L V A L L Q
76	TTTGATGGCCCCAGTCACCAGAAGAAAGTG
	F D G P S H Q K K V
136	ACATGCCAGCCCAGGGAGGTGGTGGTGCCT
	T C Q P R E V V V P
196	AAACAAGTAGTGCCCAGCTGTGTGACTGTG
	K Q L V P S C V T V
256	GGCCTGGAATGTGTGCCCACTGGGCAACAC
	G L E C V P T G Q H
316	TACCCGAGCAGTCAGCTGGGGGAGATGTCC
	Y P S S Q L G E M S
376	CCTAAAAAAAAGGAGAGTGCTGTGAGGCCA
	P K K K E S A V R P
436	<u>CAGCCCCGCTCTGTTCCGGGCTGGGACTCT</u>
	Q P R S V P G W D S

Fig.9(i)

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cgttgcgctgcctgcgcccagggctcggga	
ccgccccgggtccccgggtccgcgccatgg	
ccgggctagggccccgATGAGCCCCCTGCTG	
M S P L L	-17
CTGGCTCGCACCCAGGCCCTGTGTCCCAG	
L A R T Q A P V S Q	4
GTGCCATGGATAGACGTTTATGCACGTGCC	
V P W I D V Y A R A	24
CTGAGCATGGAACATCATGGGCAATGTGGTC	
L S M E L M G N V V	44
CAGCGCTGTGGTGGCTGCTGCCCTGACGAT	
Q R C G G C C P D D	64
CAAGTCCGAATGCAGATCCTCATGATCCAG	
Q V R M Q I L M I Q	84
CTGGGAGAACACAGCCAATGTGAATGCAGA	
L G E H S Q C E C R	104
GACAGGGTTGCCATACCCACCCGTTCCC	
D R V A I P H H R P	124
ACCCCGGGAGCACCTCCCCAGCTGACATC	
T P G A P S P A D I	144

Fig.9(ii)



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496	<u>ATCCATCCC</u> ACTCCAGCCCCAGGATCCTCT
	I H P T P A P G S S
	S P R I L
556	CTGACCCCCGGACCTGCCGTTGCCGCTGTA
	L T P G P A V A A V
	P D P R T C R C R C
616	GGGGCTTAGAGCTCAACCCAGACACCTGTA
	G A *
	R G L E L N P D T C
676	ctttccagactccacgggcccggctgcttt
736	agcacaggcgtaacctcctcagtctgggag
796	gagctctctcgccatctttttatctcccaga
856	atgtctcacctcaggggccaggggtactctc
916	ttctggctggctgtctcccctcactatgaa
976	gggttctgttatgataactgtgacacacac
1036	gacactaaaaaaaaaaaaaaaaaaaaaaaaa

Fig.9(iii)

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GCCCGCCTTGCACCCAGCGCCGCCAACGCC	
A R L A P S A A N A	164
C P P C T Q R R Q R	130
GACGCCGCCGCTTCCTCCATTGCCAAGGGC	
D A A A S S I A K G	184
R R R R F L H C Q G	150
↓	
GGTGCCGGAAGCCGCGAAAGTGAcaagctg	
	186
R C R K P R K *	167
tatggccctgcttcacagggagaagagtgg	
gtcactgccccaggacctggaccttttaga	
gctgccatctaacaattgtcaaggaacctc	
tcacttaaccaccctgggtcaagtgagcatc	
aaccccaaacttctaccaataacgggattt	
acacactcacactctgataaaaagagatgga	
aaaaaaaaaaaa	

Fig.9(iv)

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41/52	42/52
<i>Fig 10(i)</i>	<i>Fig 10(ii)</i>

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A

hVRF167	-21	MSPLLRRLLLLAALLQLAPAQAP	↓
mVRF167	-21	MSPLLRRLLLLVALLQLARTQAP	
hVRF167	30	EVVVPLTVELMGTVAKQLVPSC	
		:	
mVRF167	30	EVVVPLSMELMGNVVKQLVPSC	
hVRF167	80	ILMIRYPSSQLGEMSLEEHSQC	
		:	
mVRF167	80	ILMIQYPSSQLGEMSLGEHSQC	
hVRF167	130	RPDPRTCRCRCRRRSFLRCQGR	
		:	
mVRF167	130	RPDPRTCRCRCRRRRFLHCQGR	

B

hVRF186	116	RAATPHHRPQPRSVPGWDSAPG
mVRF186	116	RVAIPHHRPQPRSVPGWDSTPG
hVRF186	166	TPGPAAAAADAAASSVAKGGA*
		:
mVRF186	166	TPGPAVAADVDAASSIAKGGA*

Fig.10(i)

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VSQPDAPGHQRKVVSWIDVYTRATCQPR 29

| | | : : : | | | | | | | | |

VSQFDGPSHQKKVVPWIDVYARATCQPR 29

VTVQRCGGCCPDDGLECVPTGQHQVRMQ 79

[illegible]

VTVQRCGGCCPDDGLECVPTGQHQVRMQ 79

ECRPKKKDSAVKPDSPRPLCPRCTQH HQ 129

[illegible]

ECRPKKKESAVRPDSPRILCPPCTORRO 129

GLELNPDTCRCRKLRR\* 167

.....:

GLELNPDTCRCRKPRK\* 167

APSPADITHPTPAPGPSAHAAPSTTSAL 165

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361	362	363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390	391	392	393	394	395	396	397	398	399	400	401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421	422	423	424	425	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443	444	445	446	447	448	449	450	451	452	453	454	455	456	457	458	459	460	461	462	463	464	465	466	467	468	469	470	471	472	473	474	475	476	477	478	479	480	481	482	483	484	485	486	487	488	489	490	491	492	493	494	495	496	497	498	499	500	501	502	503	504	505	506	507	508	509	510	511	512	513	514	515	516	517	518	519	520	521	522	523	524	5
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APSPADI IHPTPAPGSSARLAPSAANAL 165

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*Fig. 10(ii)*

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<i>Fig 11(i)</i>	<i>Fig 11(ii)</i>

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mVRF167	-21	MSPLLRRLL..LLVALLQL..
		: :   :
mVEGF188	-26	MNFLLSWVHWTLALLLYLHH
mVRF167	25	TCQPREVVVPLSMELMGNVV
		:   :   : : :
mVEGF188	24	YCRPIETLVDIFQEYPDEIE
mVRF167	75	QVRMQILMIQYPSSQ.LGEM
		:       :       :
mVEGF188	74	NITMQIMRIKPHQSQHIGEM
mVRF167	119	.....ILCPPC
		:
mVEGF188	124	QKRKRKKSRLFKSWSVHCEPC
mVRF167	152	GLELNPDTCRCKPRK
		: :
mVEGF188	173	QLELNERTCRCDKPRR

Fig.11(i)

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AR.TQAPVSQFDGPSHQKKVVPWIDVYARA	24
:   : :  : :  : ::	
AKWSQAAPTT.EGEQKSHEVIKFMDVYQRS	23
KQLVPSCVTVQRCGGCCPDDGLECVPTGQH	74
:      :   :      : :        : :	
YIFKPSCVPLMRCAGCCNDEALECVPTSES	73
SLGEHSQCECRPKKKESAVRPDSPR.....	118
: :	
SFLQHSRCECRPKKDRTKPEKKSVRGKGKG	123
TQRRQR...PDPRTCRCRCRRRRFLHCQGR	151
:   :      :   : :  :	
SERRKHLFVQDPQTCKCSCKNTDS.RCKAR	172
	167
	188

Fig.11(ii)



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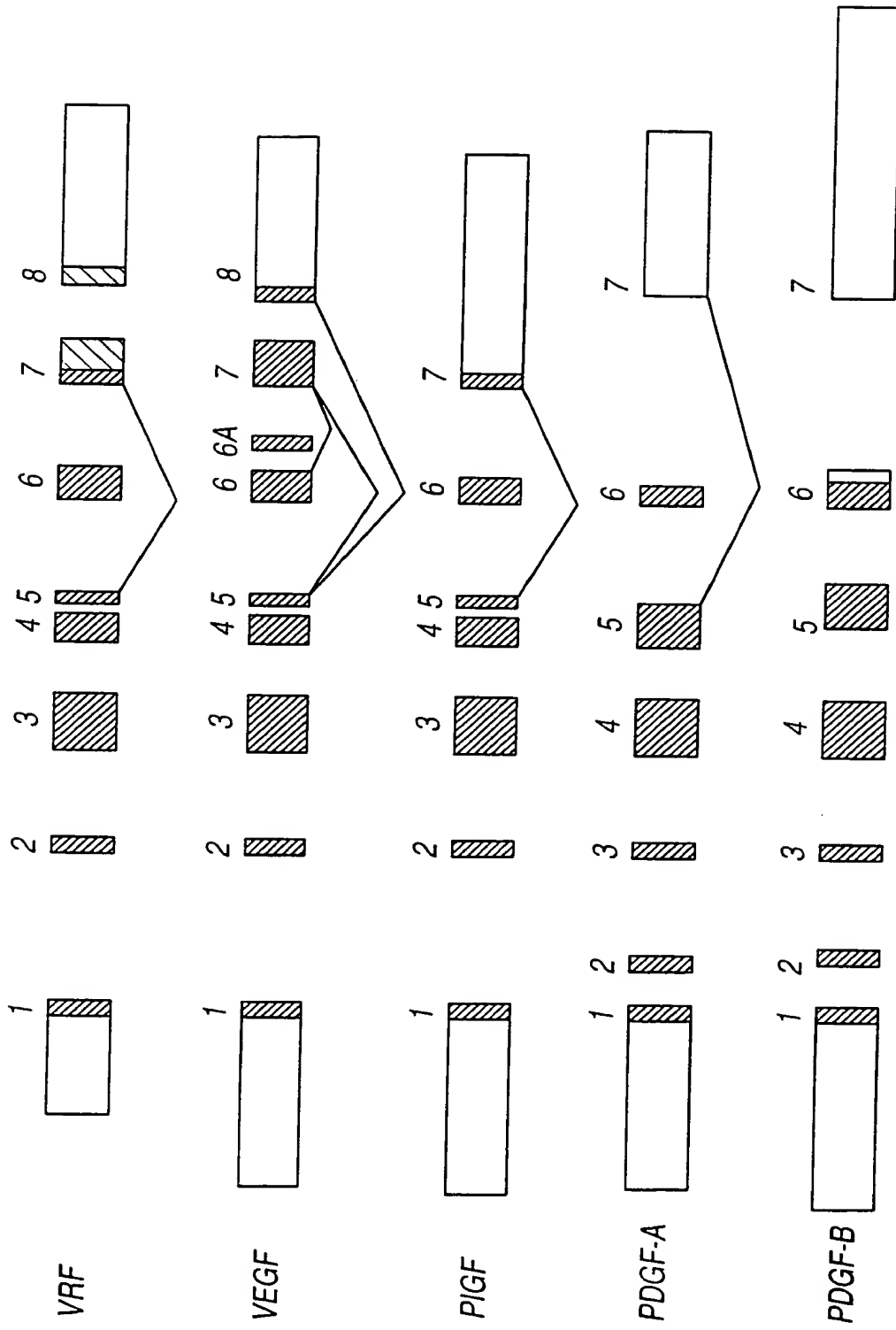
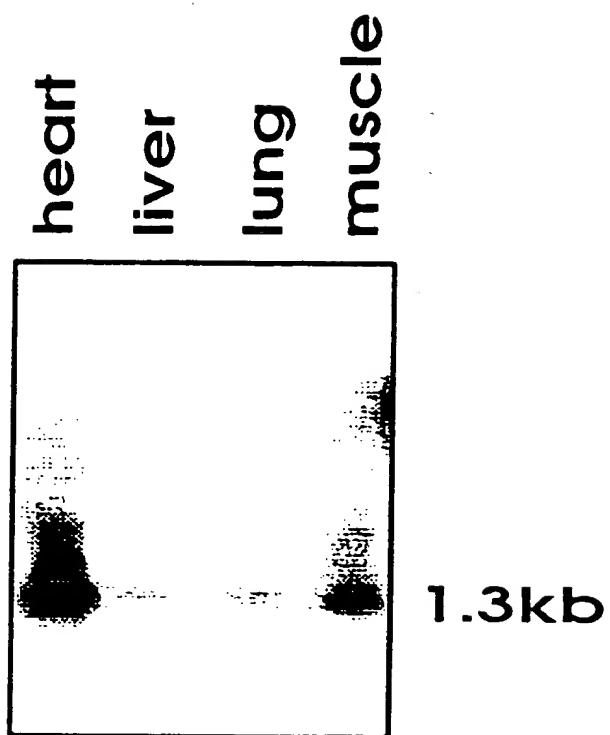


Fig.12

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*Fig.13*

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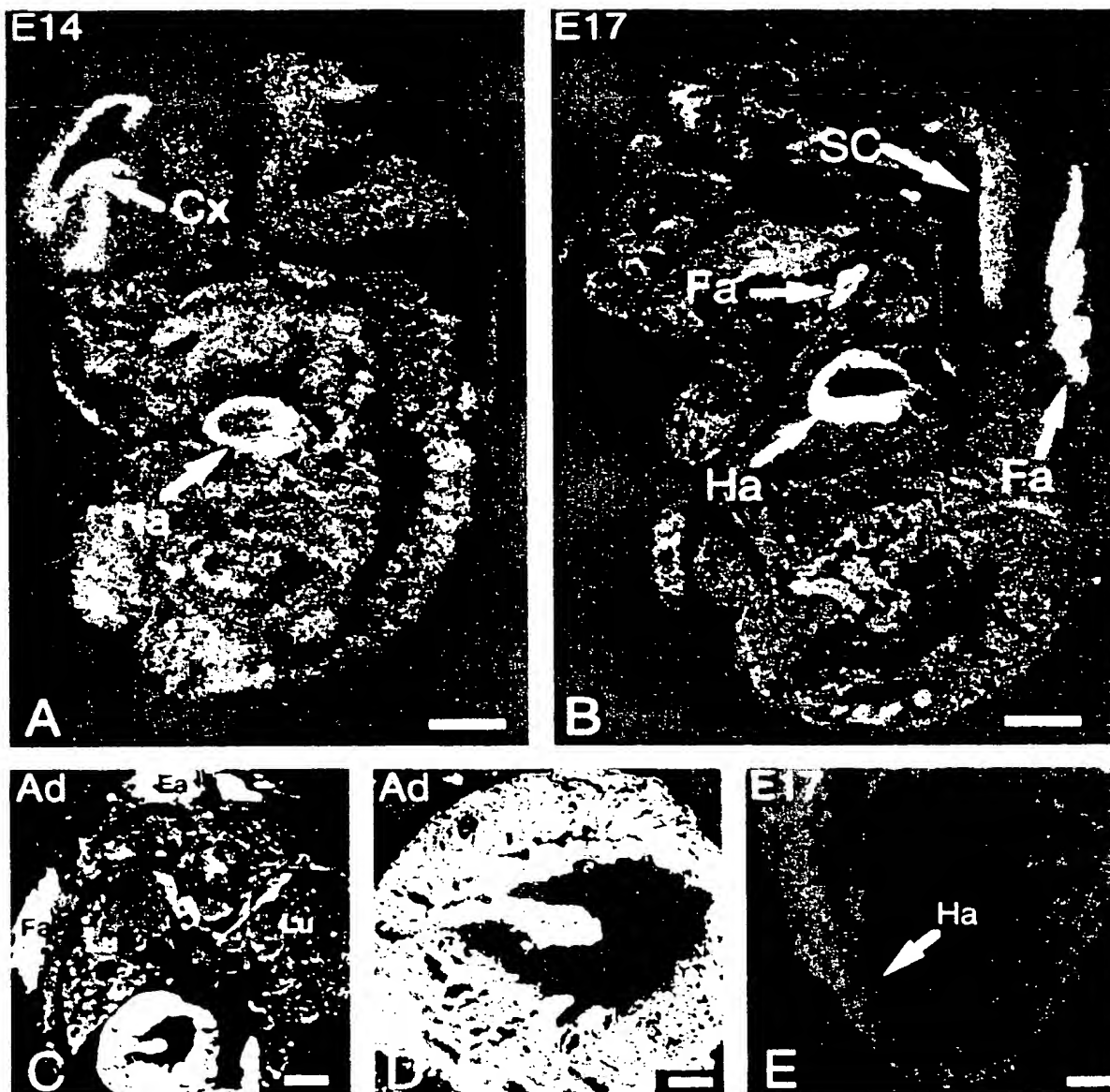


Fig.14

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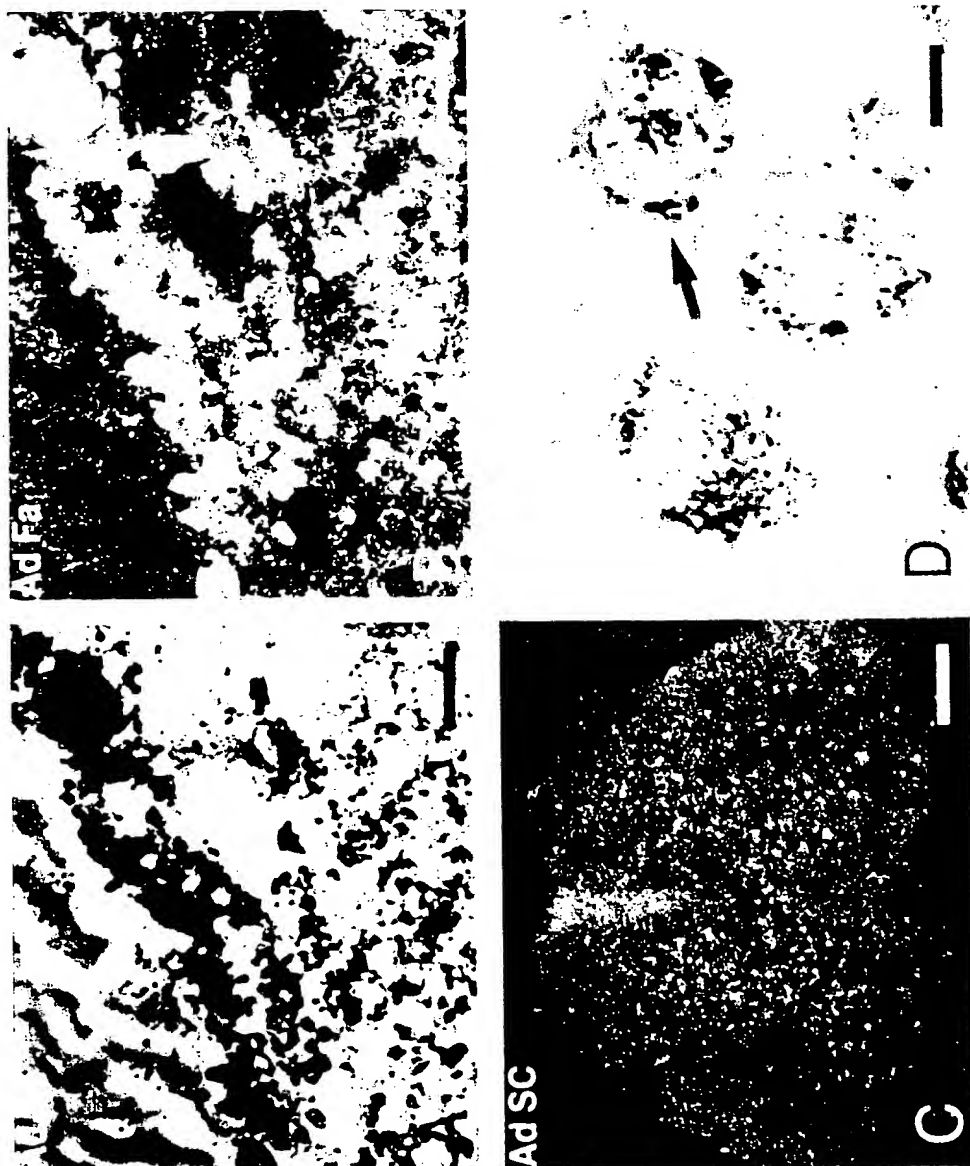


Fig. 15

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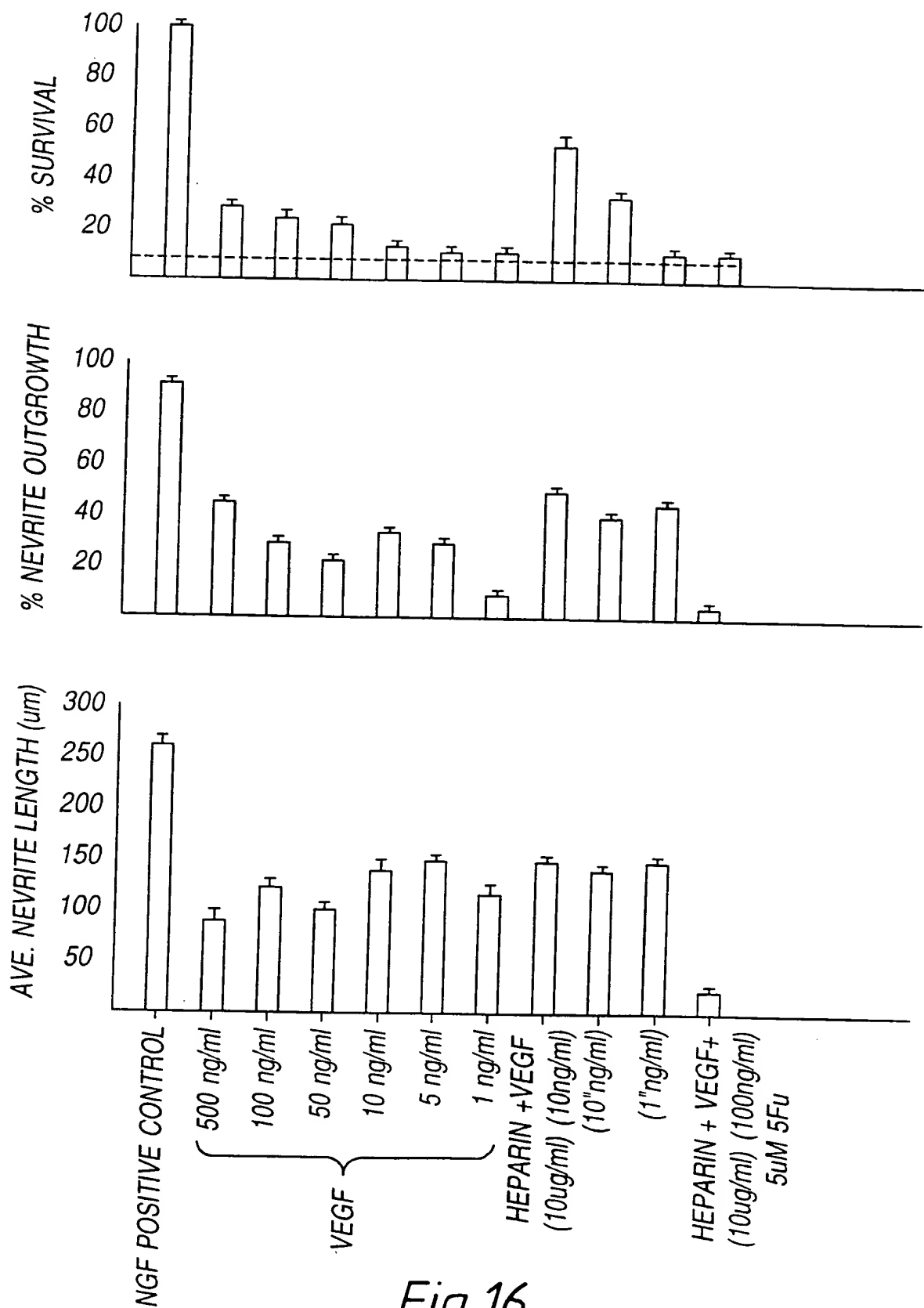


Fig.16

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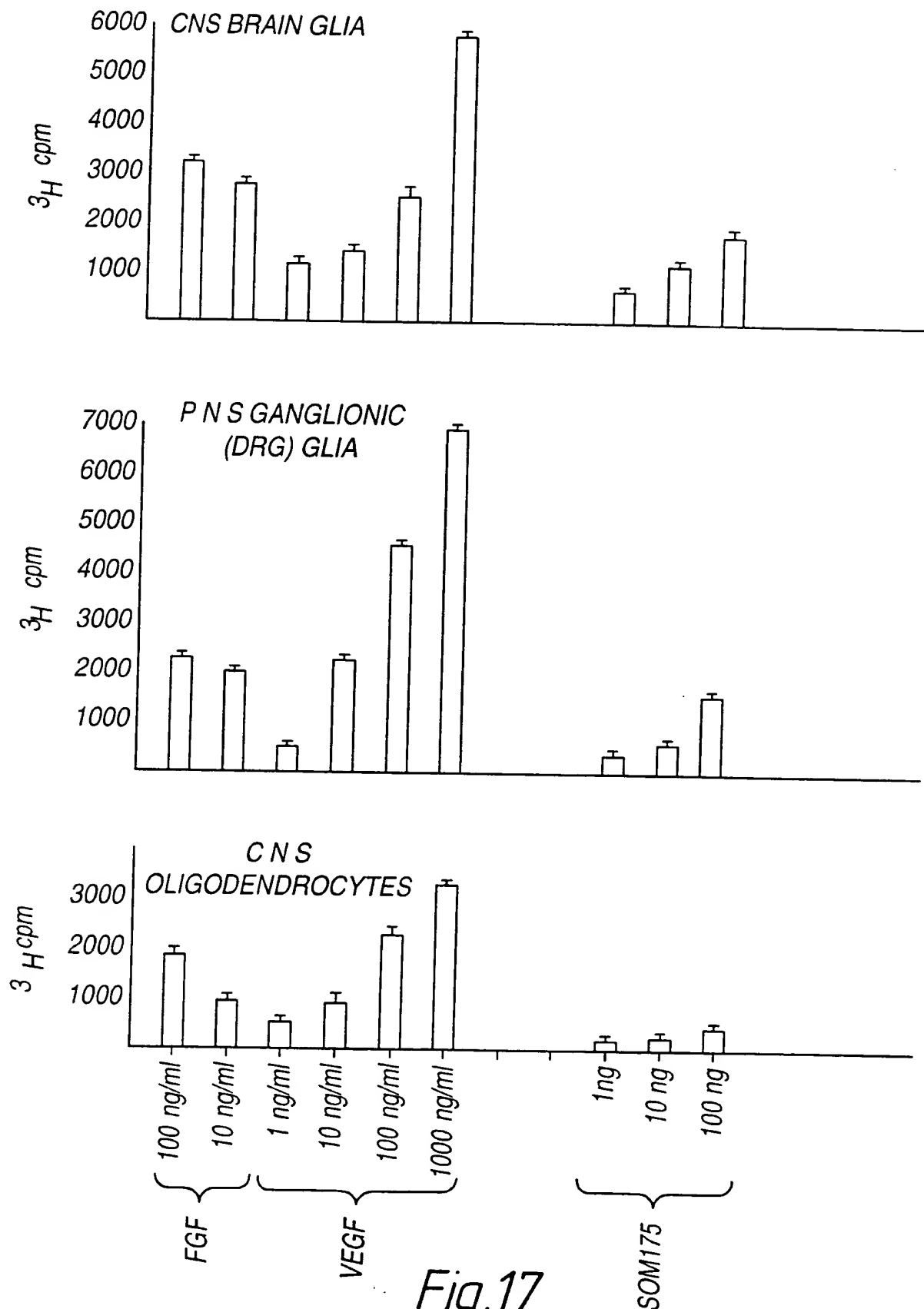


Fig.17  
SUBSTITUTE SHEET (RULE 26)

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## MOUSE ASTROGLIAL CELLS

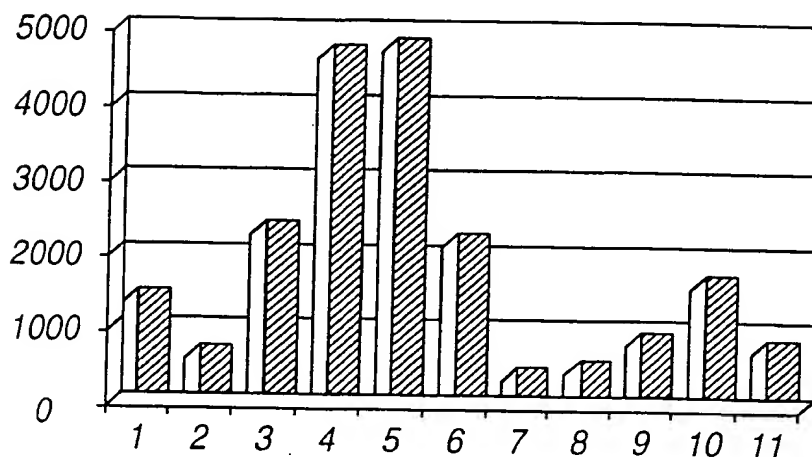


Fig.18

## MOUSE OLIGODENDROGLIAL CELLS

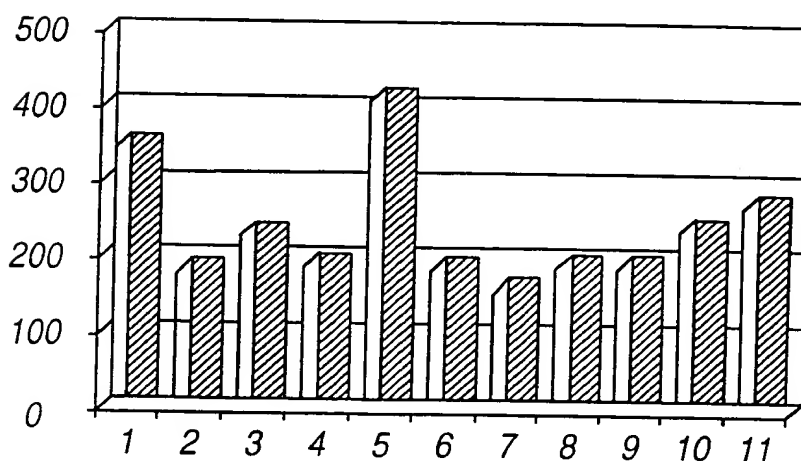


Fig.19

## MOUSE FOREBRAIN NEURONS

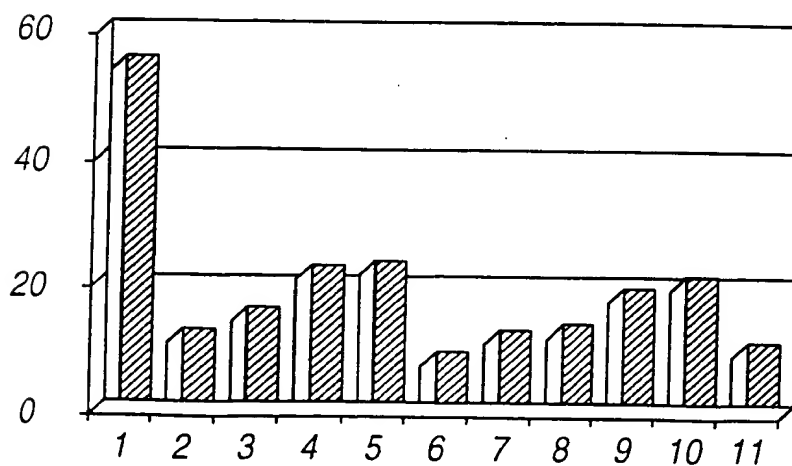


Fig.20

# INTERNATIONAL SEARCH REPORT

International Application No.

PCT/AU 96/00094

## A. CLASSIFICATION OF SUBJECT MATTER

Int Cl<sup>6</sup>: C12N 15/12; C07K 14/475; A61K 037/02

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

WPAT AND CHEM ABS

SEE DETAILS IN ELECTRONIC DATABASE BOX BELOW

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched  
USPM, JAPIO

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
DERWENT WPAT, USPM, JAPIO DATABASES; KEYWORDS: MVRF OR HVRF OR SOM 1: OR SOM X: OR [VASOACTIVE ( ) PERMEABILITY ( ) FACTOR#] OR VEGF: OR VEGF OR VRF: OR VRF OR [GROWTH ( ) FACTOR (5N) (VASCULAR OR ENDOTHELI:)]  
CHEMICAL ABSTRACTS DATABASE; KEYWORDS: AS ABOVE

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	AU 60798/90 (CALIFORNIA BIOTECHNOLOGY INC) published 21 February 1991	1-41
X	AU 56574/90 (GENENTECH, INC.) published 15 November 1990	1-41
P,X	AU 73941/94 (HUMAN GENOME SCIENCES, INC.) published 14 September 1995	1-41

☒ Further documents are listed in the continuation of Box C

☒ See patent family annex

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E" earlier document but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
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Date of the actual completion of the international search  
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## C (Continuation)

## DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5073492 (Chung-Ho Chen and Sumi C. Chen) published 17 December 1991	34
X	Biochemical and Biophysical Research Communications (1992), Vol 183, No. 3, pg 1167-1174 (Weindel K. et al.) "AIDS-ASSOCIATED KAPOSI'S SARCOMA CELLS IN CULTURE EXPRESS VASCULAR ENDOTHELIAL GROWTH FACTOR". See whole Article.	1-41
X	Biochemical and Biophysical Research Communications (1989), Vol 165, No. 3 pg 1198-1206 (Edmund Tischer et al.) "VASCULAR ENDOTHELIAL GROWTH FACTOR : A NEW MEMBER OF THE PLATELET-DERIVED GROWTH FACTOR GENE FAMILY". See whole Article.	1-41
X	Journal of Virology (1994), Vol 68, No. 1 pg 84-92 (David J. Lyttle et al.) "HOMOLOGS OF VASCULAR ENDOTHELIAL GROWTH FACTOR ARE ENCODED BY THE POX VIRUS ORF VIRUS". See whole Article.	1-41
X	Methods in Enzymology (1991), vol 198, pg 391-405 (Ferrara Napoliana et al.) "PURIFICATION AND CLONING OF VASCULAR ENDOTHELIAL GROWTH FACTOR SECRETED BY PITUITARY FOLLICULOSTELLATE CELLS". See whole Article	1-41
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P,X	Biochimica et Biophysica Acta (1995), Vol. 1260 No. 2 pg 235-9 (Sharma Hari S et al.) "NUCLEOTIDE SEQUENCE AND EXPRESSION OF THE PORCINE VASCULAR ENDOTHELIAL GROWTH FACTOR". See whole Article.	1-41
X	DEVELOPMENT (1992), Vol 114, pg 521-532 (Breier G et al.) "EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR DURING EMBRYONIC ANGIOGENESIS AND ENDOTHELIAL CELL DIFFERENTIATION". See whole Article	1-41
X	Molecular Endocrinology (1991), Vol 5 No. 12, pg 1806-1814 (Houck Keith A et al.) "THE VASCULAR ENDOTHELIAL GROWTH FACTOR FAMILY: IDENTIFICATION OF A FOURTH MOLECULAR SPECIES AND CHARACTERIZATION OF ALTERNATIVE SPLICING OF RNA". See whole Article	1-41

## INTERNATIONAL SEARCH REPORT

International Application No.

**PCT/AU 96/00094**

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

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